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HYDROSOLS OF TOTAL EGG-PHOSPHATIDES. I_A
PREPARATION AND SOME PROPERTIES OF CLEAR (HIGH DISPERSE)
AND OF LOW DISPERSE HYDROSOLS

BY

H. G. BUNGENBERG DE JONG AND J. TH. HOOGEVEEN

(Communicated at the meeting of June 27, 1959)

1. INTRODUCTION

In previous communications amphoteric phosphatide-systems have been studied as association colloids [1]. It has been shown that by diverse means the same characteristic equilibrium systems may be obtained (solution, elastic viscous solution, O-coacervate, smectic phase, P-coacervate) as are known in anionic or cationic association colloids, and further that these phosphatide systems can be realised reversibly, from one to the other, in the same sequence as in the case of anionic or cationic association colloids.

In the above studies we have intentionally paid no attention to "colloid systems of higher order" [2], that is, systems which arise by subdividing a phase into small particles. Already as a consequence of the subdivision, such phosphatide systems (e.g. hydrosols) will in principle represent non-equilibrium systems, thus masking more or less the characteristics of association colloids.

The subject of the present series of communications are colloid systems of higher order, namely disperse systems of total egg-phosphatides in water, ranging from relatively high disperse systems (clear hydrosols) to lower disperse systems. This first communication deals with the preparation of clear (high disperse) and low disperse hydrosols and some of their properties.

2. GENERAL REMARKS. NEED OF IMPROVEMENT OF THE SECOND STEP
OF THE PROCEDURE

The preparation of phosphatide hydrosols is in principle the same as used formerly [3]. The procedure may be divided into two steps.

- A) Formation of the primary sol, whereby a solution of purified phosphatides in an appropriate liquid, miscible in all proportions with water, is rapidly mixed with a sufficient quantity of water.
- B) Formation of the hydrosol by removing the original solvent from the primary sol.

As there is a tendency for the degree of dispersion of the primary sol to decrease during step B, much attention must be paid to the factors which influence this degree of dispersion. These factors, comprising the manner of purification, choice of solvent and technique of mixing, are reviewed later in section 6. It may suffice to mention that in the present communication tert. butylalcohol or mixtures of this alcohol with water are used as solvent in the formation of the primary sol, instead of ethanol.

Formerly step B was accomplished by dialysis of the primary sol (obtained from 1 vol. ethanolic solution of the phosphatide mixed with 4 volumes of water). For larger quantities of sol the dialysis is a time-consuming and impracticable procedure (e.g. several days on a Stern-dialyser, the latter placed in a refrigerator at 2° C to avoid growth of micro organisms during the long time of dialysis).

Further inconveniences are the increase in volume of the sol during dialysis and the swelling of the collodionmembrane as a result of uptake of phosphatide in the membrane.

The same or even greater inconveniences are to be expected, when in the formation of the primary sol tert. butylalcohol is used (because of the larger molecule, the dialysis will be still more time-consuming).

A method to remove the tert. butylalcohol in step B in another way is therefore desirable. It has been found that tert. butylalcohol can be removed conveniently and in a relatively short time (e.g. 1 hour or less) by a procedure which amounts in principle to a (nearly) isothermal distillation at a low temperature.

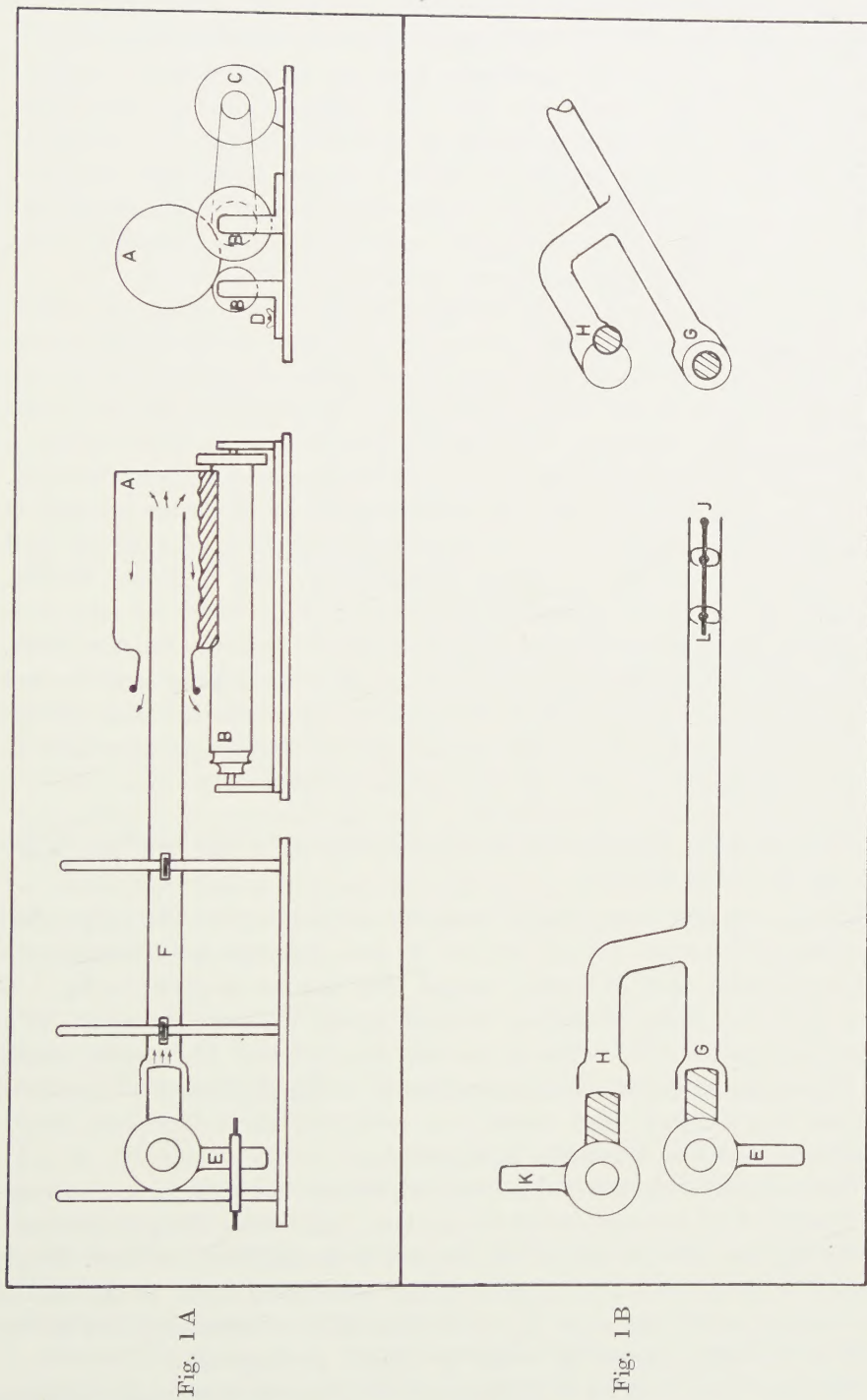
3. APPARATUS

The apparatus used is given in fig. 1, A. The cylindrical wide mouth flask A (e.g. a one liter bottle) containing primary sol (e.g. 150 ml) lies horizontally on two rubber coated rolls B, one of which is driven by a synchrone motor C. Clamps and screws D at the frame carrying the second roll allow changing the relative position in a horizontal plane of the two rolls. Thus accomodation is possible for flasks of different diameter by changing the distance of the two rolls.

The axis of the second roll also may be adjusted in such a way that the two rolls are no longer parallel to each other. This adjustment may be necessary because cylindrical flasks are often not strictly cylindrical but more or less conical.

Pulleys on the driving roll and pulleys on the axis of the synchrone motor allow us to change the rotation velocity of the cylindrical flask. In the example given the flask made 68 revolutions per minute.

Cold or hot air delivered from an electrical hairdryer (Föhn) E streams through a blow-tube F into the interior of the flask and leaves the latter through the annular space between the tube and mouth of the flask. As the efficiency of the evaporation depends among other things on the volume of transported air, the resitance for the jet of air delivered from



Blowing apparatus.

the Föhn should be as small as possible. Therefore the inner diameter of the tube (35 mM) should not be smaller than the delivering tube of the Föhn. A rigid and air-tight connection between Föhn and blow tube is not advisable and even not necessary. The tube at this end is somewhat widened and makes no contact with the delivery tube of the Föhn.

The circular space between blowpipe and inner surface of the mouth of the cylindrical flask should not be too narrow a) because then the resistance for the current of air increases b) because of diminishing the chance of contact between blowpipe and mouth of the rotating flask, the latter in general not having an ideal shape.

With the blowpipe designed in fig. 1 B, we are restricted to either blowing with cold air or with hot air. As we wished to be able to choose air temperatures in between we use a blow tube with additional sidetube as given in fig. 1, B. For each opening G and H a Föhn is operating, one delivering hot air, the other cold air. The blow tube is mounted in such a way that it can be turned sufficiently around the axis of the main tube GI.

In any position the jet of air delivered by Föhn E enters opening G of the blowtube. It depends on the relative position of Föhn K and opening H whether the jet of air delivered by Föhn K enters wholly, partially or not at all the opening H of the blowing tube. We may thus regulate the temperature of the air delivered at I into the rotating flask. In the range of lower temperatures Föhn E must deliver cool air and Föhn K hot air. In the range of higher temperature just the reverse applies. The temperature of the air at I is read off from thermometer L, fixed centrally in the tube by two spirals of wire.

4. PRELIMINARY EXPERIMENT ON THE REMOVAL OF TERT. BUTYLALCOHOL FROM A PRIMARY SOL

In this experiment we started from 150 ml primary sol (1.33 g purified egg-phosphatides per 100 ml 18.2 vol % tert. butylalcohol, present in a 1 L cylindrical flask of known weight. The apparatus given in fig. 1, A was used, the Föhn delivering cold air (room temperature about 19°). After each period of 15 minutes the flask was weighed. The loss in weight of the sol for each period has been plotted in fig. 2. The curve obtained in this way suggests that indeed in a relatively short time tert. butylalcohol is removed from the hydrosol.

When all tert. butylalcohol is removed, the loss in weight for each successive period of 15 minutes should be constant, only water being evaporated.

During the first period of 15 minutes a strong smell of tert. butylalcohol is perceived at the mouth of the cylindrical flask, at the end of the second period the smell is much diminished in intensity, and at the end of the third period it could no longer be detected.

We have made a series of dilutions of tert. butylalcohol water mixtures in test tubes and have found that the limit of the characteristic smell lies at about 0.8 vol % tert. butylalcohol.

Thus we must conclude that the steep branch of the curve corresponds to a removal of most of the tert. butylalcohol, the concentration being reduced in 45 minutes from 18.2 vol % to a value lower than 0.8 vol %.



Fig. 2. Weight loss as a function of time per quarter of an hour of the evaporating primary sol as a function of time.

It was observed that soon after the smell had disappeared the film of sol transported upward by the moving glasswall during one revolution decreases its surface considerably, as a result of which the covered part of the rotating glasswall assumes the shape of a trapeze.

This contraction (which depends on the ratio of revolution) is at the beginning of the experiment only to be observed at the mouthside of the flask (fig. 3A) but when the removal of tert. butylalcohol is far advanced a contraction begins to manifest itself also at the bottomside of the flask (fig. 3B). After the smell has disappeared, the contraction on both sides becomes strong (fig. 3C).

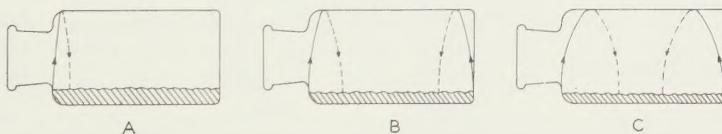


Fig. 3. Withdrawal of the evaporating phosphatide sol from the innerwall of the flask with increasing removal of tert-butylalcohol.

Thus the progressive removal of tert. butylalcohol can be roughly followed by the disappearance of the smell and the strong contraction of the liquid film on the glasswall.

Meanwhile it has been found that the limit of the smell test can be shifted to a lower tert. butylalcohol concentration. To a few ml solution is added a few g. of solid NaCl. The test tube is now closed with the finger and the tube heated just to boiling. Immediately after this the smell

of tert. butylalcohol could be detected when the original solution contained 0.2 vol % or more tert. butylalcohol. With the sol obtained at the close of the experiment this test was negative. Thus the tert. butylalcohol-concentration was lower than 0.2 vol %.

Though the experiment described in this section has proved that tert. butylalcohol can be removed almost totally by isothermal distillation at low temperature, we are not yet informed how much the loss in weight must be to reach a certain tert. butanol concentration still present in the remaining sol.

To obtain information on this point we will investigate in the next section the removal of tert. butylalcohol from a 18.2 vol % tert. butylalcohol solution containing no phosphatide.

5. REMOVAL OF TERT. BUTYLALCOHOL FROM A 18.2 VOL % TERT. BUTYLALCOHOL-WATER MIXTURE

The removal of tert. butylalcohol with the aid of the above described blowing device can be followed by means of the change in density. We therefore first determined with the aid of Ostwald pycnometers the density at 25.0° of a number of tert. butylalcohol-water mixtures ranging from 0.091 vol % to 18.2 vol % tert. butylalcohol.

The results given in Table I and plotted in fig. 4 are the means of triplo or quadruplo determinations.

TABLE I
Density of tert. butylalcohol-water mixtures at 25° C

vol % tert. butylalcohol	(d/d _{H₂O}) _{25°}	vol % tert. butylalcohol	(d/d _{H₂O}) _{25°}
0.091	0.9998 ⁸	3.03	0.9958 ⁴
0.182	0.9997 ⁰	5.20	0.9930 ⁶
0.357	0.9994 ⁶	6.83	0.9910 ⁸
0.70	0.9989 ³	9.10	0.9883 ⁶
1.03	0.9984 ⁷	11.38	0.9858 ⁸
1.35	0.9980 ⁷	13.0	0.9840 ¹
1.65	0.9976 ⁸	15.2	0.9816 ⁷
1.95	0.9973 ²	18.2	0.9781 ⁴
2.51	0.9964 ⁶		

The experiments on the removal of tert. butylalcohol from 18.2 vol % solution were made in the following way.

A large stock solution of 18.2 vol % was made and served for all single experiments. Each experiment started with 150 g of the 18.2 vol % stock solution, using air of about 55°. Separate experiments were performed for different blowing times. After blowing was stopped, the flask was cooled to room temperature and weighed. The weight of the flask itself being known we obtained the weight of the remaining liquid. Samples of the liquid were stored for determination of the density. With the aid of fig. 4 we thus find the tert. butylalcohol concentration.

The results are given in Table II. Unfortunately the flask got lost after the first 6 experiments and had to be replaced for the remaining 4 experiments by a similar flask. Minor differences in dimensions between the two flasks influenced the rate of evaporation; this follows from the fact that the weight of remaining liquid in the second flask after 35 minutes blowing is larger than in the first flask after 30 minutes blowing.

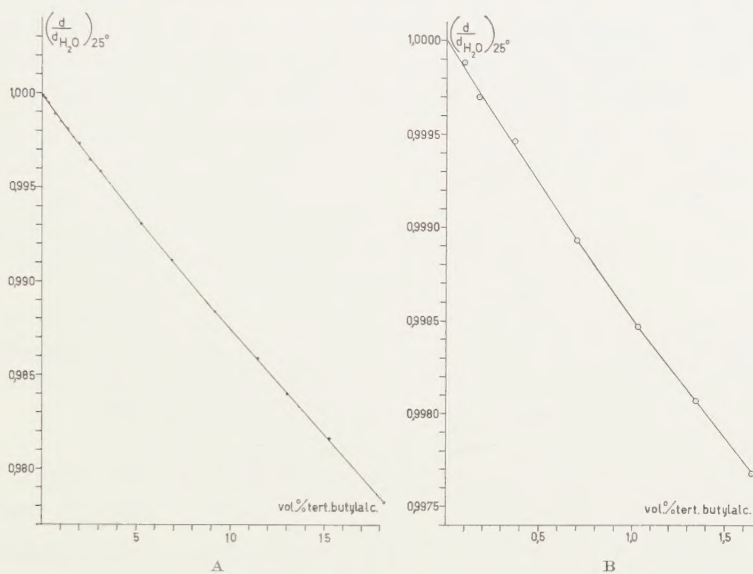


Fig. 4. Density of tert. butylalcohol-water mixtures.

There are, however, many factors which influence the rate of evaporation (e.g. room temperature, relative humidity of the air in the room, precise position of the blow tube in the rotating flask, distance between

TABLE II
Removal of tert. butylalcohol

Time of blowing	Weight of remaining liquid	$(d/d_{H_2O})_{25^\circ}$ mean of duplo	tert. butylalcohol concentration (vol. %)	100 $\frac{\text{weight remaining in liquid}}{\text{weight original liquid}}$
0'	150	0.9781 ⁴	18.2	100
5'	134.49	0.9863 ⁶	10.95	89.7
10'	124.47	0.9917 ⁸	6.3	83.0
15'	113.67	0.9958 ⁸	3.0	75.8 first 1 liter
20'	105.18	0.9982 ⁶	1.20	70.1 flask
25'	97.45	0.9990 ⁴	0.63	65.0
30'	89.81	0.9996 ³	0.24	59.9
35'	90.94	0.9996 ⁶	0.22	60.6
40'	84.87	0.9998 ⁷	0.07	56.6 second 1 liter
45'	78.93	0.9999 ⁵	0.03	52.6 flask.
60'	56.62	1.0000 ³	(-0.02)	37.8

blow tube and inner wall of the mouthpiece of the flask and capacity of the Föhn used). Therefore no general rule can be given for the removal of tert. butylalcohol in terms of time. As the procedure by which tert. butylalcohol is removed approximates a distillation, it must be expected that we will become more or less independent of many factors, when the decrease of the tert. butylalcohol concentration is correlated with the percentual loss in weight of the liquid, or as we will do, with the weight of the liquid remaining in the flask expressed in percents of the original weight of the 18.2 vol % solution. In fig. 5 we have thus plotted the values of column 4 of Table II against the values of column 5.



Fig. 5. Removal of tert. butylalcohol. The decrease in tert. butylalcohol concentration in evaporating a tert. butylalcohol-water mixture (starting with 18.2 vol % tert. butylalcohol) is plotted as a function of the weight fraction remaining.

During the blowing the curve is followed downward to the left (see arrow). The figure shows that when the weight of the liquid is reduced to 50 % of the original weight, the remaining tert. butylalcohol concentration is less than the sensitivity threshold of the two smell tests described in section 4 (0.8 vol % and with NaCl 0.2 vol %) and even approaches the experimental error of the density determination.

In fig. 6 we have plotted the logarithm of the tert. butylalcohol concentration as a function of the time of blowing (fig. 5A) and of the percentage of the weight of the remaining liquid (fig. 5B). Using the logarithm of the tert. butylalcohol concentration as ordinate we may better see what happens in the range of low tert. butylalcohol concentrations.

We see that in fig. 6A, in which the removal of the tert. butylalcohol is correlated with time, the points corresponding to flask I and flask II lie on different curves, the evaporation being slower in the case of flask II (due to minor differences in dimensions of the two flasks).

The experimental points obtained with the two flasks lie however on the same curve in fig. 6B as predicted above.

The results of the present section lead to a rule of thumb which is probably also valid if instead of a tert. butylalcohol-water mixture, a

primary sol containing a few percent egg-phosphatides is taken. According to this rule the tert. butylalcohol is practically removed when the weight of the original primary sol has been reduced by half.

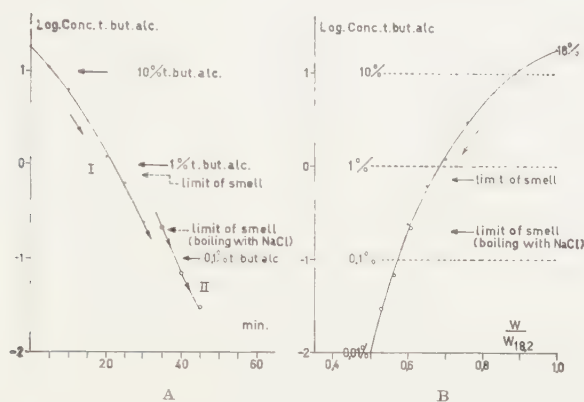


Fig. 6. Removal of tert. butylalcohol. A. The decrease in tert. butylalcohol concentration in an evaporating 18.2 vol % tert. butylalcohol-water mixture as a function of *time*, plotted logarithmically. B. The same decrease, plotted logarithmically, as a function of the weight fraction remaining.

6. FACTORS WHICH INFLUENCE THE DEGREE OF DISPERSION OF THE PRIMARY SOL

A. Clear primary sols

Some important factors which promote the formation of clear primary sols have been recognized for many years [3].

- 1) Purification of the commercial phosphatide sample by means of repeated precipitation of the ethereal solution with acetone.
- 2) Precipitation of a solution of the crude egg phosphatides in a mixture of 1 volume ethanol 95 % + 1 volume acetone by adding 1 volume of water, this precipitation preceding the purification mentioned sub 1).
- 3) The concentration of the purified phosphatide in the solvent (formerly most times ethanol 95 %) used for the preparation of the primary sol should not be too high.
- 4) Rapid mixing of the ethanol solution (e.g. 1 volume) with water (4 volumes) by injecting the solution in a fine jet in excess water stirred vigorously.

The purification mentioned sub 1) removes fats, fatty acids, cholesterol and cholesterol esters which may be present in small amounts in the commercial sample. This purification alone does not lead to clear primary sols, the product still showing a strong opalescence. It has been shown by A. DE BAKKER [4] that after threefold precipitation with acetone from the ethereal solution the phosphatides still contain aminoacids (or lower peptides). A. de Bakker has given a method to remove these. The phosphatide is dissolved in 60 vol % ethanol and is shaken with

carbon tetrachloride. The CCl_4 layer contains the phosphatides, the amino acids remaining in the ethanol-water layer. We have found that this removal of amino acids is essential for obtaining clear primary sols. In the next section two examples of the preparation of clear hydrosols are given, the first of which use this removal of amino acids.

The precipitation mentioned sub 2) obviously removes some substance(s) which hinder the formation of clear primary sols. Recent work has shown that this procedure also removes aminoacids (or peptides). It has been found that the purification sub 2) preceding the one according to the purification sub 1) also leads to clear primary sols. This combined purification is used in the second example given in the next section.

As to point 3) the use of tert. butylalcohol, combined with the removal of tert. butylalcohol by means of isothermal evaporation, has many advantages above the formerly used ethanol.

To obtain a 1 % hydrosol with the aid of formerly used methods, the primary sol had to be prepared by rapidly mixing a 5 % phosphatide solution in ethanol with 4 volumes of water. It is not possible here to use an alcohol-water mixture as solvent, because over a broad range of alcohol concentrations egg phosphatides are not soluble. Contrary to this, egg phosphatides are soluble in 30 vol % tert. butylalcohol at room temperature; and besides the formation of sols is much easier here.

To obtain a 1 % phosphatide hydrosol we may for instance start from a 1 % solution of phosphatides in 30 vol % tert. butylalcohol and mix this solution rapidly into 1 volume of water. The 0.5 % primary sol containing 15 vol % tert. butylalcohol is then subjected to isothermal distillation with the aid of the blowing device, until its weight is reduced to half the original value. We thus obtain a 1 % hydrosol.

For the rapid mixing (point 4) we use the apparatus given in fig. 7. The solution of phosphatide in 30 vol % tert. butylalcohol in vessel A is injected through the bent capillary B into beaker C, provided with the chosen quantity of distilled water, by means of compressed air delivered by flask D. Before the injection is started by opening stopcocks E and F, the magnetic stirring device G is set into operation. The stirring rod H provides for an intense stirring, the formation of a vortex of air downward to the centre of the liquid being prevented by a number of vertical ribs protruding into the interior of the beaker (see I).

B. Lower disperse "primary sols"

By modifying one or more of the factors mentioned above in an unfavourable way, lower disperse primary sols are obtained.

One might for instance omit the removal of fatty contaminants and (or) the removal of aminoacids or peptides. One might also choose another solvent (e.g. ethanol) instead of a tert. butylalcohol-water mixture. One might also combine these unfavourable factors. Since we limit ourselves for the time being to systems which are analytically of the same

type, we omit consideration of the factors mentioned, and consider only factor 4 mentioned above, that is the rapidity with which the 30 vol % tert. butylalcohol solution of the purified egg phosphatides is mixed with water.

For obtaining lower disperse "primary sols", the solution of the purified phosphatides in 30 % tert. butylalcohol is brought in a beaker and the required amount of water is added very slowly (drop for drop) from a burette, while it is stirred slowly by means of a magnetic stirring device. In this way the critical range of concentrations of tert. butylalcohol in which the clear solution changes into a colloidal system is passed slowly. The nature of the colloidal systems formed between 30 and 20 vol % tert. butylalcohol have been studied in previous communications [5, 6]. First, an elastic viscous system is gradually formed. With more water the system loses its elasticity, leaving a strongly opalescent primary sol.

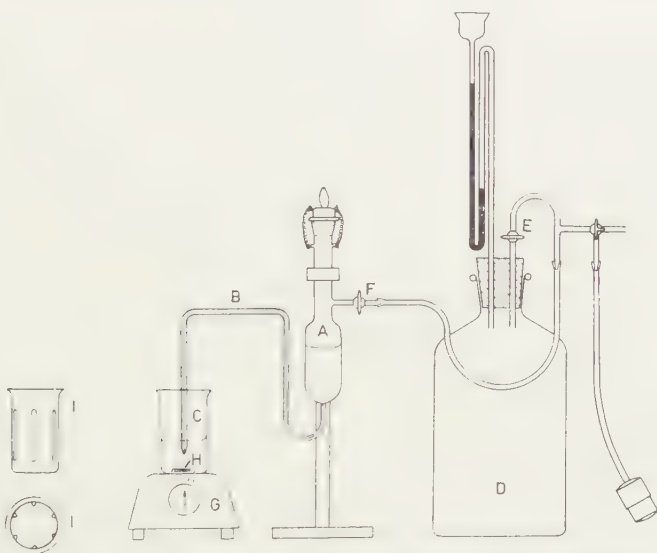


Fig. 7. Injection apparatus for preparing high-disperse primary sols.

7. EXAMPLES OF THE PREPARATION OF CLEAR HYDROSOLS

A. Removal of amino acids or peptides following the purification with ether + acetone

Starting material was fresh "Eilecithin pur." (Merck), which after arrival had been stored in a CaCl_2 exsiccator at -10°C . In a one liter medicine flask 5 g crude egg phosphatides and 30 ml ether are brought, after which the flask is closed with a cork. After solution is complete, 75 ml acetone is added under agitation. The precipitated mass is allowed to settle on the wall, the closed flask lying horizontally on the table. The supernatant liquid can after some time be poured off from the precipitated mass adhering to the glass wall. The flask is closed and put

for some time vertically on the cork, by which the precipitated mass is sufficiently drained. The small volume of supernatant liquid is poured off and discarded. The precipitated mass is dissolved in 25 ml ether and precipitated with 62.5 ml acetone. Again one waits until the flocculated mass adheres to the glass wall, continuing as already described.

For the third and last precipitation the phosphatide is dissolved in 20 ml ether and is precipitated with 50 ml acetone, etc. The drained precipitated mass is now dissolved in a small quantity of ether and the solvent is slowly evaporated, while the flask is in a rolling movement on the rolling device of fig. 1. As ether rapidly evaporates, we should not use an airstream, for then the temperature of the ether will decrease below the dewpoint of the air and water will condense on the phosphatide.

We only warm the outside wall of the flask with a Föhn, thus preventing the contents of the flask to cool down too far.

After evaporation of the ether, the phosphatide is present as a thin film on the wall.

We now dissolve this film in 37.5 ml CCl_4 and pour this solution into a separating funnel and add 42.5 ml "60 vol % ethanol" (60 volumes ethanol 95 % + 40 volumes H_2O). After shaking separation into two layers takes place rapidly, but it is necessary to wait some time (e.g. $\frac{1}{2}$ hour) before both layers are perfectly clear. The layers are separated and the ethanol-water layer is discarded. The CCl_4 layer is shaken for a second time with the ethanol-water rich layer of a "blank separation", obtained by shaking 37.5 ml CCl_4 with 42.5 ml "60 vol % ethanol". In this way the separation into two clear layers remains rapid (when instead of this procedure we had shaken the phosphatide containing CCl_4 rich layer with 42.5 ml "60 vol % ethanol", the separation would be difficult).

The CCl_4 layer is now brought into a wide mouth flask and the solvent is evaporated by means of the blowing device. As the CCl_4 layer contains besides phosphatides also some ethanol (which is as easily removed as CCl_4) and water (which is removed slowly), we do not at once obtain a film of phosphatides on the glass wall. While the contents in the flask are still liquid, phosphatides separate. From now on the evaporation is very slow and it makes no sense to continue the vaporation. We now add into the flask some molten tert. butylalcohol, which dissolves the phosphatides, and begin anew to evaporate while blowing. When a white precipitate appears anew after blowing for some time, once more tert. butylalcohol is added, and so on, until all phosphatide is present as a clear thin layer on the glass wall.

It would now be possible to dry the phosphatide on the glass wall to constant weight (the weight of the cylindrical flask being known), but this is not preferable because we must blow a long time (e.g. 1 hr) with hot air (e.g. 60°) until constant weight is reached. It has been found that by this procedure the phosphatides are changed (e.g. the surplus negative

charge increases). It is, therefore, better to dissolve the film in 91 vol % tert. butylalcohol and store this solution in the refrigerator. As we know from experiments that after the above purification the yield amounts to about 75–80 % of the original phosphatide, we can make a solution of roughly the desired concentration, while the precise concentration (g per 100 ml) can be determined on a sample of this stock solution by a dry weight determination.

From the stock solution we may then prepare the solution necessary for the preparation of the primary sol. (e.g. a 1 % solution in 30 vol % tert. butylalcohol). By following the directions given in the preceding sections we obtain a 1 % phosphatide hydrosol. This sol must be stored in the refrigerator or preferably be used at once for experiments.

B. Removal of amino acids or peptides preceding the purification with ether+acetone

The crude phosphatide (5 g) is dissolved in 95 % ethanol (50 ml)+acetone (50 ml). The solution, which may be slightly turbid is filtered into a dry separating funnel. We now add 50 ml distilled water, and shake.

The precipitated phosphatides (small double refracting lumps) now collect in the foam. We allow the liquids to stand for a sufficient time, to enable the precipitated mass to cohere somewhat. Now by cautiously opening the stopcock, the liquid is let off drop for drop. In this way it is possible to remove the liquid, the cohering phosphatide mass adhering as a conical mass to the glass wall of the separating funnel. After sufficient draining of the phosphatide mass, it is dissolved in the funnel in ether and precipitated with 2.5 volume of acetone. The funnel is laid down horizontally, so that the flocculated mass settles down on the glass wall of the funnel. After waiting for some time it forms a coherent mass and the liquid can be removed. This precipitation from ethereal solution is repeated two more times. The purified phosphatide is dissolved in ether and the solution is brought in a cylindrical flask with not too wide an opening and the ether is evaporated on the rolling device. (without blowing air into the interior, but with warming the flask from the outside with hot air by means of a Föhn). The phosphatide on the wall is dissolved in 91 % tert. butylalcohol and stored in the refrigerator. For preparing a hydrosol we then follow the directions as already given above under A.

(To be continued.)

COLLOID CHEMISTRY

HYDROSOLS OF TOTAL EGG-PHOSPHATIDES. IB PREPARATION AND SOME PROPERTIES OF CLEAR (HIGH DISPERSE) AND OF LOW DISPERSE HYDROSOLS

BY

H. G. BUNGENBERG DE JONG AND J. TH. HOOGEVEEN

(Communicated at the meeting of June 27, 1959)

8. THE BEHAVIOUR OF CLEAR HYDROSOLS TOWARDS LOW CONCENTRATIONS OF URANYL AND LONG CHAIN ALKYL-TRIMETHYL AMMONIUM SALTS. CONTRAST WITH LOWER DISPERSE HYDROSOLS

For the sake of brevity we will in this section and the next distinguish between "rapid sols" and "slow sols", which terms will denote that, in preparing the primary sol, a range of tert. butylalcohol concentrations in which sol formation takes place, is passed rapidly or slowly. For egg phosphatides, purified as described in section 7, this range lies at room temperature between about 26 and 21 vol % tert. butylalcohol. The critical range is passed rapidly, if the primary sol is prepared by injecting a solution of purified phosphatides in 30 vol % tert. butylalcohol in a fine jet into water while vigorously stirring (see section 7A and fig. 7). The primary sol is then clear, and a practically clear hydrosol is obtained after removal of the tert. butylalcohol.

The critical zone is passed slowly when we follow the method described in section 7B, the water being added slowly to the solution of purified egg phosphatides in 30 vol % tert. butylalcohol. The primary sol and the hydrosol obtained from it has then a strongly opalescent appearance.

From the difference in opalescence one may already conclude that the rapid sols are much higher disperse systems than the slow sols. But to say that rapid and slow sols only differ by the degree of dispersion is certainly too simple a statement.

From former investigations regarding what happens in the above-mentioned critical range (microscopical investigations (5) and measurements of the change of the electrical resistance (6)) it may be concluded that in rapid sols the phosphatide particles are more or less spherical, while in slow sols they consist of structured fragments, the structure units being plates or thin films.

The above must be taken into account to explain the differences in behaviour of rapid and slow hydrosols towards low concentrations of uranyl salts (acetate or nitrate) and towards long chain alkyl-trimethyl ammonium salts (bromides, the longchain alkyl group being for instance cetyl or linoleyl).

An example of the behaviour of a rapid hydrosol towards uranylacetate is given in Table III. We first prepared (in test tubes) 0.48 ml uranylacetate solutions of increasing concentration by means of varying amounts of uranylacetate solution of 2 meq/l (column 1) and of water (column 2).

TABLE III

Transient turbidity of a clear sol at low uranyl acetate concentrations

ml uranylacetate 2 meq./liter	ml H ₂ O	First 1 ml 2 % sol added	Second 1 ml 2 % sol added
0.04	0.44	turbid → opalesc.	no turbidity, only opalesc.
0.08	0.40	turbid → opalesc.	no turbidity, only opalesc.
0.12	0.36	turbid → opalesc.	no turbidity, only opalesc.
0.16	0.32	turbid → opalesc.	no turbidity, only opalesc.
0.20	0.28	turbid → opalesc.	no turbidity, only opalesc.
0.24	0.24	turbid → opalesc.	no turbidity, only opalesc.
0.28	0.20	turbid → opalesc.	no turbidity, only opalesc.
0.32	0.16	turbid → opalesc.	turbid → opalesc.
0.36	0.12	turbid → opalesc.	turbid → opalesc.
0.40	0.08	turbid → opalesc.	turbid → opalesc.
0.44	0.04	turbid → opalesc.	turbid → opalesc.
0.48	—	turbid → opalesc.	turbid → opalesc.

Then we added 1 ml of a clear 2 % egg phosphatide sol. It could be observed that during the pipetting a more or less strong turbidity originated, which within a very short time disappeared completely, leaving a slight opalescence which is about the same as shown by a mixture of 0.48 ml H₂O + 1 ml sol (see column 3 of Table III).

This transient turbidity can easily be overlooked. A more convenient way to observe it, is to add the sol very slowly, the point of the pipette being in contact with the inner wall of the test tube. The sol lies as a supernatant layer on the 0.48 ml uranylacetate solution, see fig. 8A. When the tube is now slightly agitated, both layers are mixed and a more or less strong turbidity appears (fig. 8B), which disappears spontaneously

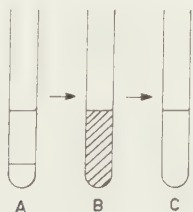


Fig. 8



Fig. 9

Fig. 8. Appearance of test-tubes when the sol and uranyl salt solution layer are mixed (transient turbidity).

Fig. 9. Mushroom-like appearance of a drop of clear phosphatide sol, entering into an uranyl salt solution from an injection needle (observed under the microscope).

within 1–2 seconds (fig. 8C). After having added 1 ml 2 % sol to each tube of the series we add once more 1 ml 2 % sol. The initial, rapidly disappearing turbidity no longer occurs in all tubes but (in the given example) from the eighth tube on, see Table III, column 4.

We may conclude that the first ml 2 % hydrosol has taken away practically all uranyl ions in the first seven tubes, but that from the eighth tube on more uranyl ions are present than can be bound by the phosphatide present in 1 ml 2 % hydrosol. Taking the uranyl ions present in 0.30 ml uranylacetate (2 meq l) equivalent with one ml of 2 % hydrosol, one finds that in the given example 33300 g of phosphatide can bind one equivalent of uranyl ions. The equivalent weight of total egg phosphatides thus arrived at, is of the same order of magnitude as that found in an earlier investigation on the reversal of charge with hexonitrate, where a value of 20000 was found [7].

The surplus negative charge of egg phosphatides has been ascribed to the presence, in small amounts, of an intrinsically negatively charged substance which cannot easily be removed from the phosphatides by the purification methods used. Without claiming here that this substance must be phosphatidic acid (as was formerly proposed) we will call it here the “negative component”.

We now turn to the behaviour of slow hydrosols towards the salts mentioned in the title. If we prepare a new series of test tubes containing increasing amounts of uranylacetate and if we add to each 1 ml 2 % slow hydrosol we find a totally different behaviour as compared with the rapid hydrosol. A turbidity zone at once appears, in the middle of which soon a permanent flocculation of practically all phosphatide will occur.

This maximum flocculation takes place in concentrations as low, approximately, as found above with rapid hydrosols. The underlying cause must be again the binding of UO_2 ions by the “negative component” present in the total phosphatides. Recapitulating we have the following striking difference:

Rapid Sol	transient turbidity, but no flocculation of the phosphatide
Slow Sol	permanent flocculation zone, practically all phosphatide present taking part in the flocculation.

9. DISCUSSION OF THE CONTRAST BETWEEN RAPID AND SLOW HYDROSOLS

To explain this striking difference we must take into account the different shape of the sol particles in rapid (spherical) and slow sols (structured fragments). The behaviour of the slow hydrosols can be explained when it is assumed that during the formation of the primary sol the negative component is built into the developing plate-like phosphatide structures.

The negative component, which has very likely an amphipatic nature,

is assumed to be taken up with its hydrocarbon chain parallel to the hydrocarbon chains of the phosphatide molecules, its negatively ionized group lying at the surface of the very thin structures. The particles of the slow hydrosols thus consist of fragments of these structures carrying a surplus negative charge at their surface.

When instability of the slow sol occurs as a result of the binding of UO_2 ions to the charged groups, necessarily all phosphatide will be involved in the flocculation and there is no reason why the flocculation should be other than permanent ¹⁾.

The behaviour of the rapid hydrosols, on the contrary, indicates that the molecules of the negative component do not form part of the spherical phosphatide particles. They may be present in a free state, or more probably loosely adherent on the surface of the phosphatide particles. Therefore, when an equivalent amount of uranyl ions is bound to the negative component, no permanent flocculation of all phosphatide present will occur.

At first sight one is then inclined to explain the initial turbidity as a flocculation of the free negative component with UO_2 ions. This interpretation raises difficult questions, viz. a) why the initial turbidity is so strong (taking into account that expressed in molecule percent its concentration amounts to only 1 or 2 % of the phosphatide), b) why one does not observe a distinct maximum of turbidity in the series of tubes and, last not least, c) why this turbidity disappears so rapidly.

These difficulties led us to investigate microscopically what happens when the rapid sol comes into contact with an uranyl salt solution.

A small cuvette is filled with uranyl acetate 0.002N. A syringe filled with the clear hydrosol is dipping with its needle point in the solution. With a horizontally placed microscope we now observe what happens when a very small quantity of sol is delivered from the needle point. The sol drop apparently does not mix with the uranyl salt solution. A sharp boundary is visible at first between the two solutions. The drop itself assumes a peculiar shape, somewhat like a mushroom with long narrow stem, the latter being connected with the needle point. This shape may remain visible up to some 20 seconds, during which its contours become less and less sharp, until it can no longer be observed.

These microscopical observations inform us 1) that no flocculations occur in any stage 2) that, when sol and uranyl salt solution are in contact, a membrane is formed, which is not stable and disappears gradually. As a result of 1) we no longer need to consider the difficulties mentioned above sub a), b) and c).

Point 2 gives an explanation of the real cause of the transient turbidity when gently agitating a test tube in which a sol layer lies supernatant

¹⁾ After long standing the flocculation zone shifts into the direction of higher UO_2 -acetate concentrations. This phenomenon will be studied in the following communication of this series.

on a uranyl salt solution. The membrane between the two layers is broken mechanically and now a very great number of new membranes is formed, which leads to total reflection of the light. After the total reflection has reached a maximum it disappears because mechanically the membranes are constantly broken until sol and uranyl acetate solution have been totally mixed.

The existence of a membrane at the boundary of rapid sol and uranyl salt solution leaves open the two above-mentioned possibilities for the negative component in the clear rapid sol. If this component were present in a free state, the membrane could be a coagulation membrane of the negative component with UO_2 ions. Taking into account the originally sharp boundary surface and the subsequent instability of the membrane, the other possibility, namely that the negative component might be very loosely attached to the phosphatide particles, appears probable. When just formed, the membrane might consist of the UO_2 salt of the negative component, loosely attached to phosphatide particles. Soon both uranyl and negative component are separated from the phosphatide, and a small coagulate of the negative component with UO_2 ions remains, escaping observation.

When we review the explanations that may account for the contrast between rapid and slow hydrosols there still remains a difficulty.

In the case of slow sols (relative) stability has been ascribed to the surplus negative charge on the surface of the particles, due to the firmly built in negative component. Neutralisation of this surplus charge, by binding an equivalent amount of UO_2 ions, removes this surplus charge. The particle surface now carries an equal amount of positive and negative charges, as a result of which flocculation sets in.

In the case of rapid hydrosols, however, the explanation given for the initial, soon disappearing turbidity, presupposes that sol particles consisting only of phosphatide molecules, having on their surface equal numbers of positive and negative charges, do not coalesce.

Supposing for a moment that we had succeeded in removing the negative component from the egg phosphatides, the following statement would summarize our conclusion.

It will not be possible to obtain stable slow hydrosols of pure egg phosphatides, whereas stable rapid sols of pure egg phosphatides may be realized. Since the surface of the particles in both cases consists of a mosaic of equal amounts of positive (choline or ethanolamine groups) and negative charges (phosphate groups) it may seem contradictory that the two kinds of sol behave in different ways.

If, however, we take into account the shape of the particles in slow and rapid sols, the contradiction becomes understandable. In the case of slow sols the particles consist of structured fragments, consisting of plates or thin films. Here plates or films of adjacent particles may fit together with their charge mosaics over considerable areas.

In the case of rapid sols the particles are (approximately) spherical. Here the charge mosaics on adjacent particles can fit together only over a small fraction of their surface. Conceivably thermal movement breaks the contacts between spherical particles, and thus a stable sol would be possible. The energy of thermal movement may not be sufficient to destroy contacts between slow sol particles, because here the contact areas are much larger.

SUMMARY

1. An improved method of preparation of clear hydrosols of total egg phosphatides has been described in detail.

2. The improvements consist in a better purification of the crude phosphatides, the choice of a tert. butylalcohol-water (30 vol %) as solvent for the purified phosphatides instead of ethanol, and the removal of tert. butylalcohol from the primary sol by means of a technique which amounts to a nearly isothermal distillation of the primary sol, the latter being obtained by injecting rapidly the 30 vol % tert. butylalcohol solution in an equal volume of water.

3. Formerly the purification consisted only in a three times repeated precipitation of phosphatide from ethereal solution with acetone. The improvement consists in a subsequent removal of amino acids (or peptides) by extracting the phosphatides dissolved in CCl_4 with 60 vol % ethanol. The amino acids (peptides) go into the alcohol-water-rich layer, the phosphatides remain in the CCl_4 layer.

4. An alternative way of removing amino acids (and peptides) consists in precipitating the crude phosphatides, dissolved in 1 volume ethanol 95 % + 1 volume acetone, with 1 volume of water. The precipitated phosphatide is subsequently thrice precipitated with acetone from an ethereal solution. The removal of amino acids by this two-stage method leads to much clearer hydrosols than the purification with ether + acetone only.

5. In 30 % tert. butylalcohol phosphatides are soluble. Our new method permits us to take a lower concentration of phosphatides in the solution which rapidly mixed with water gives rise to the primary sol than in the case of ethanol. This is of advantage as a low phosphatide concentration of the solution injected into water is favourable for obtaining clearer primary sols. A further advantage is that tert. butylalcohol gives better primary sols (other factors being equal).

6. The removal of alcohol from the primary sol was formerly accomplished by dialysis. This time-consuming and impracticable method (several days on a Stern dialyser at 2°), has now been improved by means of a device in which air delivered from one or two Föhns (electrical hairdryers) is blown through a wide tube into the interior of a rotating cylindrical flask containing the primary sol.

7. The removal of tert. butylalcohol from a 18.2 vol % tert. butylalcohol has been followed by means of density measurements. From these experiments follows a convenient rule: When by evaporation the weight of the remaining sol has been reduced to half the weight of the original primary sol, the tert. butylalcohol has been practically removed.

8. Low-disperse (highly opalescent or somewhat turbid) hydrosols are obtained when the solution of purified egg phosphatides in 30 % tert. butylalcohol is mixed with water in such a way that the tert. butylalcohol concentration is lowered very slowly.

(To the phosphatide solution water is added drop by drop from a burette).

9. Clear (high-disperse) hydrosols behave in a peculiar way towards uranyl salts or long chain alkyl-trimethyl ammonium salts at low concentrations. A strong turbidity occurs which disappears within 1-2 seconds, without flocculation of the phosphatide. Microscopical investigation reveals that an instable membrane is formed at the contact boundary of clear sol and salt solution. The reason for the initial turbidity is the formation of numerous membranes when the solution is stirred, visible by total reflection.

10. The low-disperse sols behave towards the same salts in low concentration in a quite different way, a permanent flocculation zone being formed.

11. The primary cause of the phenomena mentioned sub 9 and 10 is the same, namely that the cation of the salts mentioned (for instance UO_2) is bound by a negative amphipatic substance (phosphatidic acid?) which in small concentrations (a few mol per cent) is present in egg phosphatides.

12. Taking into account the results of former investigations, it is inferred that the quite different consequences of the binding of the said cations (e.g. UO_2) to the negatively charged component, when we compare high-disperse sols (8) and low-disperse sols (9), must result from:

- a) difference in shape of sol particles (in high-disperse sols spherical particles in low-disperse sols structured fragments like plates or thin films).
- b) difference in location of the negative component (in high-disperse sols this component occurs in a free state or very loosely attached to the surface of the spherical particles, while in low-disperse sols it is part of the plates or thin films).
- c) difference in stability of both types of sols in the absence of a negative component. (High-disperse sols are stable, low-disperse sols are unstable; the difference is explained by the different shape of the particles.)

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SPOT TESTS FOR PHOSPHOLIPIDS AND THEIR USE IN PAPER CHROMATOGRAPHY

BY

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1. INTRODUCTION

For paperchromatographic analysis of phospholipids either impregnated or non-impregnated filterpapers have been used by many investigators. AMELUNG and BÖHM [1] used non-impregnated papers, MARINETTI and STOTZ [2] and LEA *et al.* [3] used silicic acid-impregnated papers (WHATMAN) and HÖRHAMMER *et al.* [4] used SCHLEICHER and SCHÜLL no. 2043^b papers impregnated with formaline. The most commonly used identification reactions for phosphatides are the following: Phosphate with ammoniummolybdate; choline phosphatides with phosphomolybdic acid and SnCl_2 ; Ethanolamine phosphatides with ninhydrine (also serine phosphatides); Rhodamine G 6 or Rhodamine B; most lipid substances become visible under U.V. light; J_2 is used as a general identification reagent for all lipids; with the reagent of Schiff (fuchsin discoloured with sulphurous acid) especially aldehydes are identified; some authors use Nile Blue, Malachite Green, Sudan III or oxidizing reagents, such as permanganate or OsO_4 , as a more or less general identification reagent for lipid substances.

In this paper some new identification methods will be described: The tricomplex staining method, a phosphate staining method with the reagent of Zinzadze, the CS_2 Ag_2S staining method for primary and secondary amines, and the tricomplex Brilliant Green staining method. The new methods will be compared with methods already in use and the sensitivity and the group specificity will be discussed.

2. EXPERIMENTAL

A. Materials

Phosphatides are prepared according to RHODES and LEA [5] using Al_2O_3 columns and silicic acid columns starting from Egg Lecithin puriss. from Merck. Phosphatidyl-choline and phosphatidyl-ethanolamine are obtained in a rather pure form. Meanwhile it was possible to prepare a

reference solution containing phosphatidyl-choline, phosphatidyl-ethanolamine and their lysoproducts and sphingomyeline, in relative proportions, suitable to make paperchromatograms which enabled us to study the effect of the different identification methods. Moreover this same solution served as a general reference mixture for paper chromatographic analysis.

Further we used a commercial phosphatide mixture from soy-beans.

B. Spot-test

The spot-tests are made on filterpapers of a quality in use for paperchromatography. We have chosen Schleicher and Schüll's no 2043^b filterpaper. Most experiments are done on both non-impregnated and silicic acid-impregnated filterpapers. The impregnation is carried out as described by MARINETTI and co-workers [2, 6]. For the spot-test the solution under investigation is brought up with a special pipette (ONRUST [7]) which fills itself by capillary forces with a contents of 4.7 mm³.

The circular spots which are formed on the paper have a diameter of 9 mm.

C. Paperchromatography

Chromatograms are made according to MARINETTI and STOTZ [2]. Some modifications are proposed which lead to a considerably quicker procedure and to better separations. Results obtained with Schleicher and Schüll no. 2043^b papers were more reproducible than those obtained with Whatman no. 1 papers. By using chromatographic tanks of 8.1 liter (27 × 30 × 10 cm) and 1.9 liter (25.5 × 8.3 × 9 cm) and using impregnated papers of 24.5 × 12.6 cm (for the 8.1 liter tank) and 24.5 × 6.3 cm (both tanks), it is possible to reduce the time necessary for obtaining a good separation of the phosphatides to 4–5 hours instead of 20–24 hours as prescribed by Marinetti and Stotz. Equilibration previous to chromatography is not necessary and even leads to less succesful separations. The R_f values of the different phospholipids are strongly dependent on the distance from the starting line to the solvent front. Small differences in the distance between the starting points and the level of the mobile phase in the chromatographic tank are almost without effect on R_f values. The differences are even smaller with Schleicher and Schüll paper than with Whatman paper. The solvent used is di-isobutylketone (Schuchardt b.p. 168.1° C)–acetic acid–water 40 : 25 : 5 (vol : vol : vol). After finishing the chromatographic run the paper is dried in a stream of warm air.

3. DESCRIPTION OF THE NEWLY DEVELOPED IDENTIFICATION METHODS AND SOME CONVENTIONAL METHODS

A. Tricomplex staining

This method was described in detail by H. G. BUNGENBERG DE JONG and G. R. VAN SOMEREN in a previous article [10].

Reagents: a 50 ml 2 % Uranyl nitrate $\cdot 6\text{H}_2\text{O}$ in water
 50 ml 0,2 % Acid Fuchsin in water or 5 ml 0,2 % Acid
 Fuchsin in water
 50 ml 0,1 n HCl
 made up with distilled water to 500 ml.

The resulting solution, containing 0,02 or 0,002 % Acid Fuchsin, 0,2 % Uranyl nitrate and 0,01 n HCl with a $\text{pH} = 2$ serves as the *staining solution*.

rinsing solution

50 ml 2 % $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ in water

50 ml 0,1 n HCl

made up with distilled water to a volume of 500 ml.

Procedure:

The paper ready to carry out a spot test, or the dry chromatogram, is immersed in the staining solution for at least three quarters of an hour and next to that immersed in the rinsing solution which is renewed after 10 minutes. After another 10 minutes the paper must be dried in a stream of warm air to prevent bleeding effects. Where the phosphatides are expected to be present, a red spot will develop, more intense in colour as the amount of phosphatides is greater.

The principles of this staining are given in detail in the paper by H. G. BUNGENBERG DE JONG and G. R. VAN SOMEREN already mentioned.

B. Phosphate reaction

Reagents:

Solution A. 120 ml concentrated sulphuric acid are boiled with 6,02 gr. molybdic acid. When all the molybdic acid has been dissolved the solution is allowed to cool. 70 ml distilled water are added and after cooling the solution is made up to a volume of 200 ml.

Solution B. 0,56 gram Mo powder is added to solution A and the mixture is softly boiled. While solution A is colourless, it will get a dark blue colour after dissolving the Mo.

Staining solution.

One volume of solution B is diluted with one volume of distilled water.

Rinsing solutions.

1e. The above staining solution ten times diluted with distilled water.

2e. A saturated solution of aluminum sulphate.

Procedure:

The paper ready for the spot test or the chromatogram is immersed in the staining solution for 15 minutes, after which the paper is immersed

for 10 minutes in rinsing solution no. 1 and next for 10 minutes in rinsing solution no. 2. After 10 minutes, rinsing solution no. 2 must be renewed. This is repeated after 10 minutes. The paper must be dried in a stream of warm air.

This method is based on a micro determination for phosphate as described by ZINZADZE in 1932 [8]. The sulphuric acid of the medium hydrolyses the phosphatides so that the phosphate can react with the molybdic acid, resulting in the formation of molybdenum blue. The rinsing procedure is very important, because if this is not done with care the paper as a whole will soon be coloured blue and desintegrate.

It is sometimes necessary to outline the blue spot with a pencil when the chromatogram is still in the staining bath because we often see the blue colour disappear after the chromatogram is dried.

C. Dithiocarbamate reaction on primary amino groups (cephaline reaction)

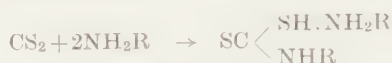
Reagents: 1e. freshly prepared mixture of 1 ml carbondisulphide and 2 ml triaethylamine.
2e. A mixture of 50 ml silvernitate, 50 drops of nitric acid (concentrated; density = 1.40) and 10 ml 95 % tert-butyl-alcohol.

Procedure:

At the bottom of an empty exsiccator is placed a little Petri disk with 1 ml CS_2 and 2 ml triaethylamine. The paper ready for the spot test or the chromatogram is placed in the upper part of the same exsiccator and must stand out freely in the vapour of the mixture of carbondisulphide and the amine. After a period of one hour or longer the paper is removed and is dried in a stream of warm air, for instance by hanging above the central heating or with a Föhn, till all the carbondisulphide and triaethylamine are removed. This will last about 20 minutes. Next the paper will be spread out over a filterpaper which is thoroughly wetted with the AgNO_3 reagent. The reaction is allowed to take place in a period of about 15 minutes. This part of the procedure should preferably be carried out in the dark. The paper is then immersed in distilled water to which is added a little $\text{UO}_2(\text{NO}_3)_2$ solution. This operation also is performed in the dark. The rinsing procedure will take at least 15 minutes and must be repeated several times to prevent the whole paper from becoming coloured black. This will occur when the AgNO_3 is not completely removed by the rinsing procedure. After rinsing the paper is carefully dried in a stream of warm air. At the places where $-\text{NH}_2$ containing phosphatides (phosphatidylethanolamine and its lysocompound) are located a dark brown colour will be visible.

This method is based on the conversion of the primary amine to a dithiocarbamate (FEIGL [9]). Dithiocarbamates of aliphatic amines are

formed almost instantly by the action of carbon disulphide on free primary and secondary aliphatic amines



The dithiocarbamates which are formed by this reaction may be detected after removing the excess carbon disulphide. The dithiocarbamate can be revealed by the formation of silver sulphide (brown colour) when silver-nitrate in acid medium is allowed to react with the reaction-product.

By performing the reaction in the vapour of the CS_2 reagent it is possible to prevent that disturbing phenomena do occur. For instance we are independent of the solubility of any chromatographed substance in the reagent-solution.

It was not possible to perform in the vapour-phase, the next step in the method, which means the conversion of the di-thiocarbamate. Therefore we developed the described procedure of spreading out the paper (chromatogram) on a thoroughly wetted filterpaper. The silvernitrate in this paper makes possible the formation of silversulphide as a reaction-product of the di-thiocarbamate with silvernitrate.

D. Brilliant-Green — Acid Fuchsin staining method

Mixed staining for phosphatides and other phospholipids.

Reagents: Staining solution:

0.006 % acid Fuchsin, 0.01 % Brilliant Green, 0.2 % $\text{UO}_2(\text{NO}_3)_2$ and 0.01 n HCl, in distilled water.

Rinsing solution:

[1] as described for the tricomplex staining method.

[2] 1 n acetic acid containing 0.2 % $\text{UO}_2(\text{NO}_3)_2$.

Procedure:

The manipulations are quite the same as prescribed for the tricomplex staining method but it is advisable to stain for a much longer time. Overnight staining is necessary to obtain the maximum intensity of the green colour, which will develop with Brilliant Green at certain places at the paper.

Rinsing with solution [1] is not satisfactory because if the chromatogram is dried the HCl will discolour the Brilliant Green. Therefore the HCl must be washed out with acetic acid; the latter is removed by drying and does not give rise to such a low pH as HCl does by drying which might be the cause of the discolouring of the Brilliant Green.

Brilliant Green is a basic dye and will stain acid substances. However, in combination with UO_2 and acid fuchsin, phosphatidylcholine and phosphatidylethanolamine their lysoproducts and sphingomyeline will preferably be stained with acid fuchsin and Uranyl. Substances with only an acid function provided the acidic group is still sufficiently ionised at

pH=2, will only take up the Brilliant Green. For more details on the mechanism of this reaction see H. G. BUNGENBERG DE JONG and G.R. VAN SOMEREN.

E. Rhodamine 6G staining

Rhodamine 6G can be added in a 0.001 % concentration to the tri-complex staining solution for the purpose of obtaining a greater contrast and to detect various lipids besides phosphatides of the type which give a colour with Acid Fuchsin and Uranyl ions (phosphatidyl-choline and -ethanolamine, their lysoproducts and sphingomyeline). The paper is viewed under Ultra Violet light.

F. Phospho-molybdic acid staining

Reagents: 1e. 2 % phospho-molybdic acid in distilled water
2e. freshly prepared 0.4 % stannouschloride in 3 n HCl.

Procedure:

A paper (teststrip or chromatogram) is immersed for 2 minutes in solution 1. Rinsing in running tap water for a period of at least 2 hours is followed by immersing the paper in the stannouschloride solution. A blue colour develops where choline-containing substances will be présent.

The method is thought to be specific for choline-containing substances (see, however, section 4).

G. Ninhydrin method

Reagent: A solution of ninhydrin 0.2 % in acetone with 5 % acetic acid.

Procedure:

The strip (teststrip or chromatogram) is immersed for a very short time in the ninhydrin solution. After removing of the acetone the paper is heated in an oven at 80° C for 10 minutes or longer when needed for detecting amino-groups containing phosphatides. On chromatograms no colour was obtainable (see also section 4).

4. SPECIFICITY OF THE REACTIONS

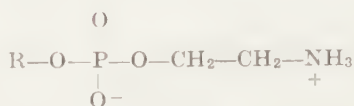
The phospholipids investigated by us are the following:

phosphatidyl-choline
lysophosphatidyl-choline
phosphatidyl-ethanolamine
lysophosphatidyl-ethanolamine
sphingomyeline

and a commercial phospholipid from soy-beans containing, among others, some of the above-mentioned substances and phosphoinositides.

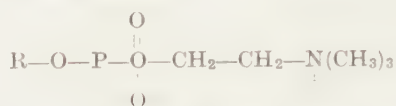
The tricomplex staining procedure has a positive effect only with phosphatides containing a basic group (choline, ethanolamine) in the molecule next to a phosphoric acid group.

On theoretical grounds we expect that only phosphatides with an amphion character will be stained with the Acid Fuchsin and uranyl-nitrate in acid medium. The amphion character is illustrated by the next formulae.



basic group: ethanolamine

Phosphatidyl-ethanolamine and
Lyso phosphatidyl-ethanolamine



basic group: choline

Phosphatidyl-choline,
Lyso phosphatidyl-choline and
Sphingomyelin.

We could prove this in the case of lecithin (phosphatidyl-choline) and cephalin (phosphatidyl-ethanolamine) with the pure substances by means of spottests. Moreover in the case of these compounds their lysoproducts and sphingomyelin, the expectation was confirmed by experiments on paperchromatograms. A very important condition for this reaction is the presence in the staining solution and rinsing solution of Uranyl-ions and the pH of the medium being sufficiently low. Without Uranyl-Acid Fuchsin does not or hardly stain the mentioned compounds at all.

The *phosphate reaction* mentioned in section 3 sub B. gives a positive staining-result with all the compounds which stain red with the tri-complex staining method, because of the fact that these contain a phosphate group within the molecule. Of course a positive staining result will also be found with other phosphate containing lipids, for instance phosphatides of the phosphoinositide type and the compounds known as phosphatidic acids (Cardiolypin). We chromatographed a crude phospholipid from soy-beans, see figure, which contains two phosphoinositides. We were indeed able to detect two spots on the chromatograms, which give a blue colour with the above method, and which did not give a colour with the tricomplex staining method. Further investigations on this point are in progress.

The *dithiocarbamate reaction* should be positive only with phosphatides containing a primary (or secondary) amino group. The ninhydrin reaction in our experience did not give reliable results with phosphatides containing a primary amino group (phosphatidyl-ethanolamine). Ethanolamine containing phosphatides such as phosphatidyl-ethanolamine give a very clear spot test with the dithiocarbamate test. On chromatograms

phosphatidyl-ethanolamine and its lysoproduct gives a brown colour with the method, as we could demonstrate. Sphingomyelin. Phosphatidylcholine and its lysoproduct do not react at all. It is possible that Phosphatidyl-serine would probably give a positive reaction, but we could not confirm this expectation since this substance was not available to us.

Brilliant Green—Acid Fuchsin staining.

Any substance containing a negative group can react with Brilliant Green and so a green colour will develop on a chromatogram where an acid substance is located. In our method at pH=2 the paper will not bind much Brilliant Green. This mixed combined Brilliant Green—Acid Fuchsin staining procedure differentiates on chromatograms between substances which contain negative groups and substances with both positive and negative groups close together. The latter are stained with Acid Fuchsin and uranylions at pH=2 and the substances with only a negatively charged group are coloured with Brilliant Green (green spots), for instance the phosphoinositides and phosphatidic acid, fatty acids and other substances. More details about this method are described in the paper mentioned already by H. G. BUNGENBERG DE JONG and G. R. VAN SOMEREN 1959 [10].

The Rhodamine 6G staining is used especially for chromatograms as an addition to the tricomplex staining solution. By viewing the chromatogram under Ultra Violet light it is possible to locate the tricomplex spots more accurately because the contrast between the dark red stained spots and the paper background is heightened. Hence it is possible to see fluorescent spots of other lipids on the paper with good success. It is a general staining method for lipids.

The phospho-molybdic acid staining procedure (see section 3F) in our experience did not differentiate sufficiently between choline-containing and ethanolamine-containing phosphatides. Ethanolamine-containing phosphatides reacted with the reagent as well. Even after a prolonged period of rinsing (3-4 hours) in running tap water a staining of ethanolamine-containing phosphatides occurred. The only thing to be said for this widely applied staining procedure is that perhaps ethanolamine-containing phosphatides bind less intensively than choline-containing phosphatides. Despite earlier claims the method is not specific enough to be useful.

The ninhydrin reaction supposedly useful for ethanolamine-containing phosphatides, in our experience was not reliable. Very weakly yellow-brown colours developed after heating a not impregnated spot test paper for long periods. The same colours developed when the paper was heated for a similar period, without having been immersed in the ninhydrin solution beforehand. Under the same experimental conditions there develops a rose colour on impregnated spot test papers if immersed in a ninhydrine solution.

5. SENSITIVITY OF STAINING PROCEDURES

In order to investigate the sensitivity of a given staining procedure we used the following method.

A paper strip 19 cm long is separated into parts of 2 by 2 cm as indicated in the figure. In the middle of each square of 2 by 2 cm, a phosphatidyl-choline or phosphatidyl-ethanolamine solution in chloroform-methanol (80 : 20 vol : vol) in a sequence of concentrations of 3 % 1 % 0.3 % 0.1 % 0.03 % 0.01 % 0.003 % 0.001 %, is applied with the special pipette already mentioned.

3 %	1 %	0.3 %	0.1 %	0.03 %	0.01 %	0.003 %	0.001 %
141 γ	47 γ	14.1 γ	4.7 γ	1.41 γ	0.47 γ	0.141 γ	0.047 γ

The spot-test series are made with non-impregnated Schleicher & Schüll paper and with silica impregnated Schleicher & Schüll paper, both for phosphatidyl-choline and phosphatidyl-ethanolamine. The special capillary pipette has a volume of 4.7 mm³, thus we applied the amounts of substance as indicated in the figure.

Spot-test series of the above type are stained with the staining methods described in an earlier part of this paper. The staining procedure is exactly as described in an earlier part of this article.

6. DISCUSSION OF THE RESULTS (Table 1)

Generally speaking, no difference in staining properties can be seen between impregnated and non-impregnated papers. The most sensitive staining procedure is the one with Acid Fuchsin Uranyl nitrate at pH = 2, the so called tricomplex staining. This procedure gives a good staining even when the concentration of the phosphatide solution is as low as 0.03 %. This means that with our experimental manipulations even an amount of 1.41 γ of phosphatides will give a good visible colour which can be seen without artificial viewing methods.

Longer staining times with or without rinsing after staining and adding of Rhodamine 6G to the staining solution, does not affect the sensitivity of the tricomplex staining procedure. One of us (H. G. BUNGENBERG DE JONG [10]) already has stated that the lower concentrations reach their maximum colour intensity with this method in a shorter time than higher phosphatide concentrations do. In recent investigations G. J. M. HOOCHWINKEL and H. P. G. A. VAN NIEKERK [11] have concluded from quantitative experiments with the tricomplex staining method that a linear dependence exists between Acid Fuchsin bound and phosphatide concentration. This linearity is reached for higher concentration after a considerable longer time of immersion in the staining solution than for lower concentrations. This is an observation which supports the theory regarding the tricomplex staining suggested by H. G. BUNGENBERG DE JONG [10]).

TABLE 1
Results of the staining sensitivity experiments

Staining procedure	Sensitivity		Particularities
	compound	visible to a concentration as low as	
Tricomplex staining: Section 3 sub A	both products phosphatidylethanolamine and phosphatidyl-choline	0.03 % (1.41 γ)	There is no difference between impregnated and non-impregnated papers. For both kinds of paper and acid fuchsin concentrations the sensitivity is the same.
Phosphate staining: Section 3 sub B	phosphatidyl-ethanolamine phosphatidylcholine after prolonged staining	0.3 % (14.1 γ) blue grey 0.3 % (14.1 γ) blue 0.1 % (4.7 γ)	Lecithin is stained more quickly than cephaline. After prolonged immersion in the staining solution (18 hours) a visibility may be reached to a concentration as low as 0.1 %. The spots of both substances are coloured blue.
Dithiocarbamate staining: Section 3 sub C	phosphatidyl-ethanolamine	0.1 % (4.7 γ)	Phosphatidyl-choline does not react.
Phospho-molybdic acid staining: Section 3 sub F	phosphatidylcholine phosphatidylethanolamine	0.1 % (4.7 γ) 0.3 % (14.1 γ)	Specificity doubtful.
Ninhydrin staining: Section 3 sub G	phosphatidylethanolamine	0.3 % (14.1 γ)	Phosphatidylcholine does not react at all. The test gives a different result dependent on the paper used. On non-impregnated paper a yellow colour develops, this colour develops also if the paper is not immersed in the ninhydrine solution; 0.3 % is just faintly to be seen. On impregnated papers a rose-purple colour will develop visible even to a concentration as low as 0.1 %.

The dithiocarbamate staining, sensitive enough to detect a spot made with a 0.1 % phosphatide solution or 4.7 γ phosphatidylethanolamine, is highly specific, because of the fact that phosphatidylcholine even in concentrations as high as 3 % (141 γ) does not give any colour.

The phosphate staining is specific, in the sense that soluble phosphates do not give a reaction since they dissolve in the staining solution. We did some spot tests with lower organic phosphates and were not able to find a positive reaction (blue colour). Thus we prefer our procedure over the spraying reaction for phosphate described by various authors (for instance HANAHAN [12], MARINETTI *et al.*, LEA [2, 6] *et al.*).

The phosphomolybdic acid staining for choline containing phosphatides and *the ninhydrinic staining* for ethanolamine containing phosphatides judging from our experience, are not specific or will not react at all (ninhydrine method).

7. DISCUSSION OF SOME FURTHER POSSIBILITIES WITH THE STAINING METHODS

We used the tricomplex staining method as a routine spot test in column-chromatographic separations to examine separate fractions, in order to ascertain when phosphatides appear or are no longer present. The tricomplex staining method also gives us a clear indication when it is necessary to bring a new solvent on the column. The phosphate staining can also be used for this purpose.

By staining a chromatogram first with the dithiocarbamate staining method and subsequently with the tricomplex staining method we obtain a chromatogram on which the ethanolamine containing phosphatides are stained brown and the choline containing phosphatides red. Sphingomyelin has about the same R_f value as lysophosphatidylethanolamine. Thus the two substances give one spot on the chromatogram which in the first instance will show with the dithiocarbamate a brown colour, and which upon subsequent staining with uranyl ions and Acid Fuchsin will show a distinct red colour, overlapping the brown colour, caused by the reaction of sphingomyelin with uranyliions and Acid Fuchsin. The reverse sequence of staining is used with good results also, first the tricomplex staining, by immersing only a short time in the staining solution, so that only faint red colours appear and after the paper has been dried and the spots have been outlined with a pencil, a subsequent staining according to the dithiocarbamate method. In this way one can more accurately ascertain which of the spots stainable by the tricomplex staining method contain a primary amino group.

As an example we give a few chromatograms shown in the figure. Chromatogram A gives on the left a series of spots originating from a 3 % Soybean "Lecithin" Solution applied with the 4.7 mm³ pipette. The right series of spots on chromatogram A and the series of spots on the

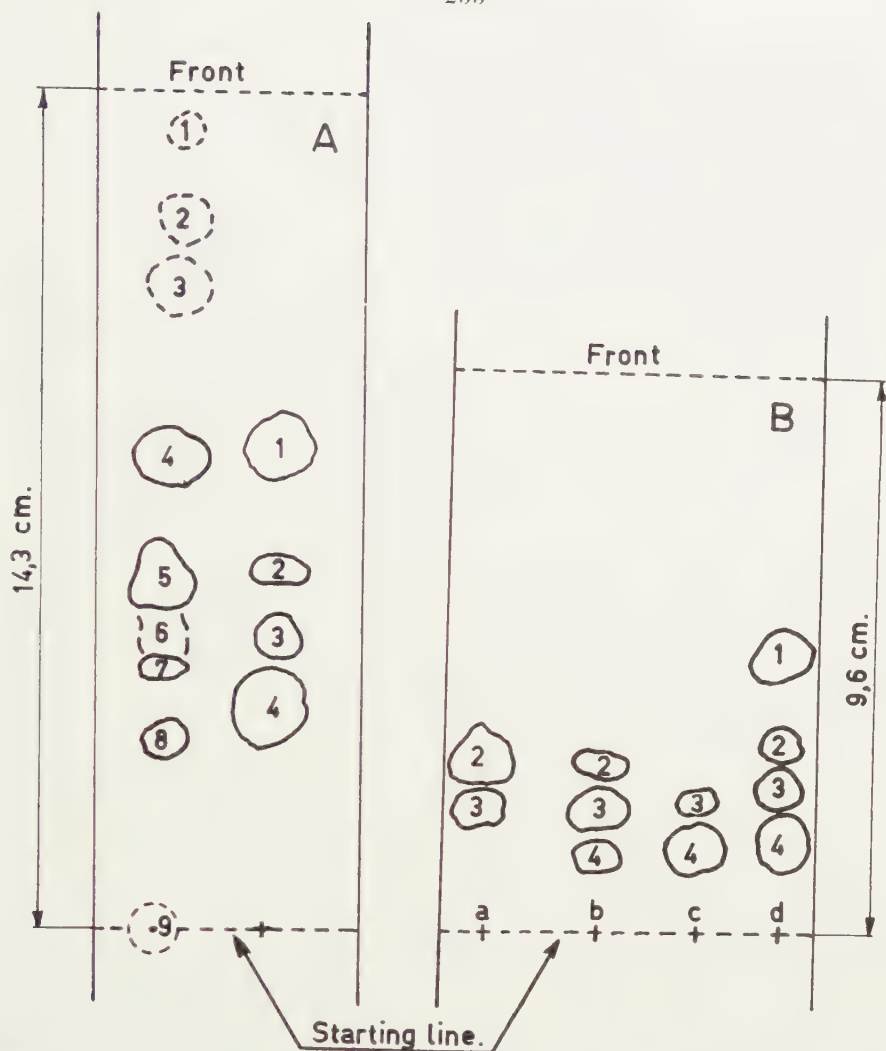


Fig. 1. The chromatograms are made with silica-impregnated S and S papers. The solvent used is a mixture of di-isobutylketone-acetic acid-water (40:25:5). These and such like chromatograms are stained with our newly developed staining methods. Details about the starting materials are given in the paper. The spots are stainable with one or more of the methods given below as indicated by the numbers in the table.

Series of spots originating from	Acid Fuchsin	Bril. Green	Phosphate	Rhod. 6. G	CS ₂ +AgNO ₃
<i>Chromatogram A</i>					
Soy-bean lecithin	4, 5, 8	1, 2, 3, 6, 7	2, 4, 5, 7, 8	1, 2, 3, 6, 7	not tested
Reference mixture	1, 2, 3, 4		1, 2, 3, 4		not tested
<i>Chromatogram B</i>					
a, b and c	2, 3, 4		2, 3, 4		
d, Reference mixture	1, 2, 3, 4		1, 2, 3, 4		1, 3

right in chromatogram B both originate from the reference mixture as described in section 2 sub A. This reference mixture contains phosphatidyl-ethanolamine (1), phosphatidyl-choline (2), lysophosphatidyl-ethanolamine (3), lysophosphatidyl choline (4) and sphingomyelin. This later substance has the same R_f value as lysophosphatidyl ethanolamine (3) and is present in the reference mixture and, moreover, is one of the components of the series of spots indicated as a, b and c on chromatogram B. In this latter mixtures are present also phosphatidyl choline (2) and its lysoproduct (4) but not phosphatidyl-ethanolamine and its lysoproduct.

SUMMARY

Some newly developed detection methods for phospholipids for spot tests and for chromatograms are given.

1. Acid Fuchsin-Uranyl ions (tricomplex-staining) method

With this method carried out in acid medium, preferably $\text{pH} = 2$, it is possible to stain phosphatides of the acid base type, for instance phosphatidyl choline phosphatidyl ethanolamine their lysoproducts and sphingomyeline. This method is based on our knowledge of tricomplex systems. Phosphatides of the acid base type are expected to stain with appropriate dyes in the presence of well-chosen ions.

The method is rather sensitive, an amount of 1.41 γ of phosphatidyl-choline or -ethanolamine present in a circular spot with a diameter of 9 mm giving a visible colour.

2. Phosphate reaction

All phosphate containing lipid spots on a chromatogram will attain a blue colour, within a few minutes if immersed in a twofold diluted stock solution as used by Zinzadze, (1932) for a colorimetric phosphate determination, prepared by dissolving MoO_3 in sulphuric acid and after addition of some water boiling with Mo. With this method even an amount of 14.1 γ phosphatidyl-choline and ethanolamine give a visible blue colour prolonged immersion in the staining solution promotes a visibility as low as 4.7 γ of the substances.

3. Reaction for primary amines

The conversion of primary amines with CS_2 in the presence of triethylamine to dithiocarbamates and the decomposition of the latter compounds with acidified AgNO_3 , leading to a brown-coloured product Ag_2S , is used as a specific detection method for phosphatides containing a primary amino group (phosphatidyl ethanolamine and its lysoproduct). The CS_2 and triethylamine are allowed to react as vapours with the dry paper, in an empty exsiccator.

Even an amount of 4.7 γ phosphatidyl ethanolamine gives a brown colour.

4. A combination of Acid Fuchsin, Uranyl ions and Brilliant Green

The above-mentioned mixture at pH=2 is used as a mixed staining solution. With this mixture it is possible to stain red, compounds of the acid-base type according to the Tricomplex principle and green acid compounds which contain a phosphate group, for instance phosphoinositides, also acidic substances, possessing other acidic groups than phosphate, e.g. fatty acids.

5. A combination of the Tricomplex staining with Rhodamine 6G

This combination results in a heightened contrast of the acid Fuchsin-stained substances on chromatograms when they are viewed in Ultra Violet light. Moreover it is possible to see under these circumstances many other lipid substances only visible in U.V. light.

The specificity of the methods is discussed, as well as their use in paper-chromatographic work and their application in spot tests to follow the course of a column chromatographic separation.

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ON THE CALCULATION OF THE MEAN EFFECTIVE PRESSURE HEAD FOR CAPILLARY VISCOMETERS

BY

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SUMMARY

When capillary viscometers are used for measurements at different rates of shear, accurate evaluation of the mean effective pressure head is required for calculation of the rate of shear. At high pressure heads an arithmetic mean value of the heads at the beginning and end of the experiment may be used, but for low pressure heads this procedure is incorrect. The situation is examined theoretically and formulae are obtained which enable the mean effective pressure head to be calculated from viscometer dimensions and measurements of the pressure head at the beginning and end of flow from the index bulb. The main case examined is for flow from a spherical bulb into a cylinder.

In measurements of liquid viscosity using capillary viscometers of the Ostwald type, the pressure head forcing the liquid through the capillary is usually taken as the excess gas pressure (if any) applied to the measuring bulb plus the pressure head of liquid in the viscometer. During an experiment, as liquid falls in one side of the viscometer and rises in the other, the pressure head is varying and for any calculations involving this a mean value must be taken. For the measurement of viscosity by comparison with a standard liquid in the viscometer it is unnecessary to know the hydrostatic head since it is the same in both cases. However, when capillary viscometers are used for measurements of viscosity at different rates of shear, the average rate of shear, $D \text{ sec}^{-1}$, can be defined by the expression:

$$(1) \quad D = \frac{\bar{H} \varrho g r}{3l\eta}$$

where $\bar{H}$ is the mean effective hydrostatic head, ϱ and η are the density and viscosity of the liquid respectively, g is the gravitational constant and r and l are the radius and length of the capillary. Thus the computation, or measurement, of the mean effective pressure head $\bar{H}$ becomes an essential feature of such experiments.

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Many authors have assumed that the mean effective pressure head can be taken as the arithmetic mean of the two extreme pressure heads, that is the pressure at the beginning and end of liquid efflux from the index bulb. It was pointed out, as early as 1881, by KOCH [1], that this assumption is, under many conditions, incorrect, the error only being negligible when the total applied head is large. Following KOCH several other authors [2-7], have also deduced formulae for the calculation of the mean effective pressure head.

In some recent work on the electroviscous effect in concentrated sols of silver iodide [8], a modified Ostwald viscometer of the type devised by FOX, FOX and FLORY [9] was employed for measurements at rates of shear over the range $200\text{--}2000\text{ sec}^{-1}$; this is illustrated in Fig. 1. In

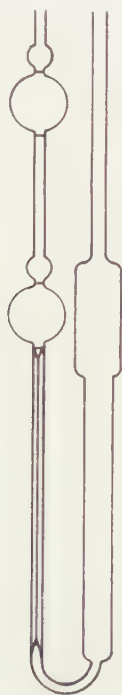


Fig. 1. Ostwald viscometer based on the design of FOX, FOX and FLORY [9].

the calibration procedure it was found that at low rates of shear, when the calibration constants were plotted against the arithmetic mean of the pressure heads the points diverged from the linear plot for pressure heads of less than 10 cm of water. Thus under these conditions it was no longer permissible to use the arithmetic mean value. Moreover, it is difficult to measure the mean effective pressure head of a viscometer experimentally, since this depends upon factors such as the length of time spent by the liquid in the lower half of the bulb in relation to that spent in the top half, although the two extreme heads can be measured accurately. Thus it is evident that the most easily accessible method is to use these measurements to compute the mean effective pressure head from a formula derived for the type of viscometer employed. The necessary

equations for this calculation, for the flow of liquid from a sphere to a cylinder, however, do not appear to have been published. The situation was therefore examined theoretically, and during this examination it became clear that a general solution could be derived, in terms of viscometer constants, which was applicable to most types of capillary viscometer. The solutions for several types of viscometer have been worked out, and in one case, flow of liquid from a sphere to a cylinder, they have been tested experimentally.

DERIVATION OF AN EXPRESSION FOR THE GENERAL CASE

To derive the general expression we will consider the case where the volumes are symmetrical about mean planes on the efflux and influx sides (Fig. 2a), namely the planes X and Y . Then if h is the arithmetic

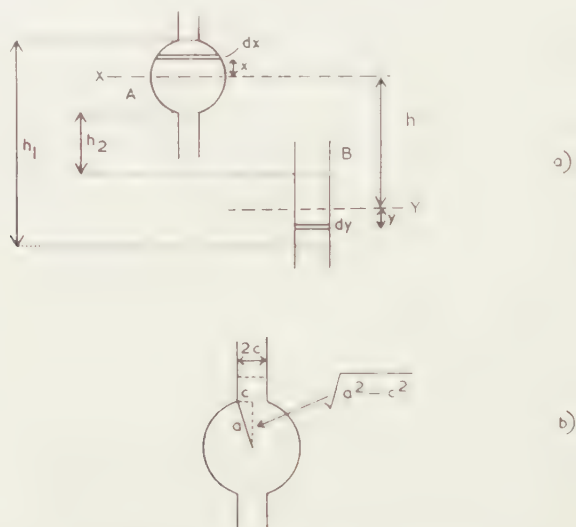


Fig. 2. a) Flow from a spherical bulb to a cylinder;
b) Correction for the volume of the neck.

mean of the pressure heads, i.e. the distance between the planes of symmetry X and Y , $\frac{h_1 + h_2}{2}$, and at an instant of time t the liquid is at a distance x above the mean line X and is at a point where the cross sectional area is A (shape of vessel not defined), then on the influx side the liquid will be at a distance y below the mean line, where the cross sectional area is B . The volumes about the mean lines are equal and hence we may write:

$$\int_0^x A \, dx = \int_0^y B \, dy.$$

When the liquid is at a point x above the plane X in the top bulb the pressure head is

$$h + x + y.$$

Therefore, the rate of flow = $k(h+x+y)$ where k is a constant, neglecting for the moment any corrections for kinetic energy effects and for resistance in other parts of the apparatus. We shall also restrict the arguments to the pressure head of the viscometer remembering that the pressure applied P , will be $(h+x+y)g$. Then if in an interval of time dt , the distance above the mean plane X changes from $x+dx$ to x and the cross sectional area is equal to A , the rate of flow is:

$$-A \frac{dx}{dt}$$

whence

$$-A \frac{dx}{dt} = k(h+x+y).$$

If x changes from d to $-d$ in time t ,

$$(2) \quad - \int_{-d}^d \frac{A}{h+x+y} dx = \int_0^t k dt = kt.$$

Therefore, for a mean effective pressure head $\bar{H}$, equation (2) can be written

$$- \int_{-d}^d \frac{A}{\bar{H}} dx = kt,$$

giving

$$(3) \quad \frac{\bar{H}}{h} = \frac{\int_{-d}^d A dx}{\int_{-d}^d \frac{A}{1 + \frac{x+y}{h}} dx}.$$

Equation (3) is a general relation, which is true even without symmetry, but for the symmetrical case we may write:

$$(4) \quad \frac{\bar{H}}{h} = \frac{\int_0^d A dx}{\int_0^d \frac{A}{1 - \left(\frac{x+y}{h}\right)^2} dx}$$

whence,

$$(5) \quad \bar{H} = \frac{h}{1 + \frac{1}{h^2} \frac{\int_0^d A(x+y)^2 dx}{\int_0^d A dx} + \frac{1}{h^4} \frac{\int_0^d A(x+y)^4 dx}{\int_0^d A dx} + \dots}$$

Thus

$$\bar{H} = \frac{\text{Arithmetic mean of the extreme pressure heads}}{\text{Correction factor}};$$

this is for symmetrical cases a general expression, and it is only necessary to solve the integral

$$(5a) \quad \frac{1}{h^{2n}} \frac{\int_0^d A(x+y)^{2n} dx}{\int_0^d A dx}$$

for the conditions involved, and to determine to what limit n has to be taken.

DERIVATION OF EXPRESSIONS FOR PARTICULAR CASES

Case 1. *Flow from a Cylinder to a Cylinder*

If the cross-sectional area of one cylinder is A and the other B we may write

$$Ax = By$$

whence by substitution in equation (3) for y , we find as A and B are constant:

$$\frac{\bar{H}}{h} = \frac{\frac{A}{h} \int_0^d dx}{A \int_0^d \frac{dx}{1 + \left(\frac{A+B}{h \cdot B}\right)x}} = \frac{2 \left(\frac{A+B}{B}\right) \frac{d}{h}}{\ln \frac{1 + \left(\frac{A+B}{B}\right) \frac{d}{h}}{1 - \left(\frac{A+B}{B}\right) \frac{d}{h}}}.$$

Thus if h_1 and h_2 are the initial and final heads,

$$h_1 = h \left(1 + \frac{A+B}{B} \cdot \frac{d}{h} \right),$$

$$h_2 = h \left(1 - \frac{A+B}{B} \cdot \frac{d}{h} \right),$$

$$h_1 - h_2 = 2 \left(\frac{A+B}{B} \right) d$$

and

$$(6) \quad \bar{H} = \frac{h_1 - h_2}{\ln \frac{h_1}{h_2}}.$$

This is MEISSNER's formula [2], and we note that this equation also applies for discharge from an open cylindrical cup into air: this is also very close to the situation in an Ubbelohde viscometer [7].

Using the general formula, equation (5), we have

$$\int_0^d A dx = Ad$$

and since

$$x + y = \frac{A+B}{B} x$$

the correction factor, considering only the first two terms, is:

$$1 + \frac{1}{dh^2} \left(\frac{A+B}{B} \right)^2 \int_0^d x^2 dx + \frac{1}{dh^4} \left(\frac{A+B}{B} \right)^4 \int_0^d x^4 dx + \dots$$

whence integrating, we have

$$1 + \left(\frac{A+B}{B} \right)^2 \frac{d^2}{3h^2} + \left(\frac{A+B}{B} \right)^4 \frac{d^4}{5h^4} + \dots$$

Thus if the diameters of the cylinders are $2a$ and $2b$, $A = \pi a^2$ and $B = \pi b^2$, giving:

$$(7) \quad \bar{H} = \frac{h}{1 + \left(\frac{a^2+b^2}{b^2} \right)^2 \frac{d^2}{3h^2} + \left(\frac{a^2+b^2}{b^2} \right)^4 \frac{d^4}{5h^4} + \dots}$$

This equation gives the mean effective pressure head in terms of the radii of the cylinders and should lead to the same answer as equation (6). For the case of two cylinders the latter equation is more convenient but as we shall see later, for bulb viscometers simple expressions of this type do not appear and the general solution becomes the more convenient.

Case 2. Flow from a Sphere to a Cylinder

This is the case to be considered for an Ostwald viscometer of the type devised by FOX, FOX and FLORY [9]. In the first instance we shall consider the case when the bulb is a perfect sphere, and then extend this treatment to the more practical case; that is, the situation when the liquid initially starts in a cylindrical neck, flows through the spherical bulb and then passes into another cylindrical tube.

2a) Flow from a Perfect Sphere to a Cylinder

If the diameter of the sphere is $2a$, and the diameter of the cylinder is $2b$, then following the previous nomenclature with $d = a$ (this means that the index mark coincides with the surface of the sphere) we may write

$$A = \pi(a^2 - x^2) \text{ and } B = \pi b^2,$$

whence

$$\pi b^2 y = \pi \int_0^x (a^2 - x^2) dx = \pi \left(a^2 x - \frac{x^3}{3} \right)$$

and

$$(8) \quad x + y = \left(\frac{a^2 + b^2}{b^2} \right) x - \frac{x^3}{3b^2}.$$

Then if we again consider the correction terms given by expression (5A), the coefficient of $1/h^{2n}$ is:

$$\frac{\int_0^a A (x+y)^{2n} dx}{\int_0^a A dx} = \frac{\int_0^a (a^2 - x^2) \left[\frac{a^2 + b^2}{b^2} x - \frac{x^3}{3b^2} \right]^{2n} dx}{\int_0^a (a^2 - x^2) dx}.$$

Substituting $x = au$ and $S = \frac{3(a^2 + b^2)}{a^2}$, then the coefficient reads:

$$\frac{\int_0^1 (1 - u^2) \left(\frac{a^3}{3b^2} (S - u^2) \right)^{2n} du}{\int_0^1 (1 - u^2) du}.$$

Since

$$\int_0^1 (1 - u^2) du = \frac{2}{3}$$

we have

$$\frac{3a^{6n}}{3^{2n} b^{4n} 2} \int_0^1 (u^{2n} - u^{2n+2}) (S - u^2)^{2n} du,$$

whence on expansion of $(S - u^2)^{2n}$ followed by integration we obtain the general term

$$\frac{3a^{6n}}{3^{2n} b^{4n}} \left[\frac{S^{2n}}{(2n+1)(2n+3)} - \frac{{}^{2n}C_1 S^{2n-1}}{(2n+3)(2n+5)} + \frac{{}^{2n}C_2 S^{2n-2}}{(2n+5)(2n+7)} - \dots + \frac{1}{(6n+1)(6n+3)} \right],$$

where ${}^{2n}C_1$, ${}^{2n}C_2$, etc. are the binomial coefficients. Thus the mean effective head is given by,

$$(9) \quad \bar{H} = \frac{h}{1 + \frac{a^6}{3b^4 h^2} \left[\frac{S^2}{15} - \frac{2S}{35} + \frac{1}{63} \right] + \frac{a^{12}}{27b^8 h^4} \left[\frac{S^4}{35} - \frac{4S^3}{63} + \frac{6S^2}{99} - \frac{4S}{143} + \frac{1}{195} \right]}$$

For a typical viscometer the capacity of the bulbs was approximately 5 ml and the measured radii of the bulbs and the cylinder were found to be 0.9 and 0.7 cm respectively, giving $S = 4.814$. From these values $\bar{H}$ has been calculated for various values of h using equation (9); the results are recorded in Table 1.

TABLE 1
Comparison between $\bar{H}$ and h for Flow from a Sphere to a cylinder

h cm	$\bar{H}$ cm		
	h	h	h
	$1 + 0.9484/h^2$	$1 + 0.9484/h^2 + 2.056/h^4$	$1 + 1.139/h^2$
30	29.97	29.97	29.97
20	19.96	19.96	19.95
10	9.92	9.90	9.89
7.5	7.38	7.38	7.35
5	4.82	4.80	4.78
3	2.71	2.66	2.66
2	1.62	1.46	1.57

It is evident from Table 1 that for pressure heads greater than 10 cm the correction is insignificant, but below this it is advisable to apply a correction. Between h values of 10 and 3 cm only the coefficient of $1/h^2$

needs to be considered but below this the coefficient of $1/h^4$ must also be included. The values of these corrections suggested that it might be possible to approximate equation (9) even further, for most of the range, and simply write,

$$(9a) \quad \bar{H} = \frac{h}{1 + \frac{a^6 S^2}{45 b^4 h^2}} = \frac{h}{1 + a^2 \left(\frac{a^2 + b^2}{b^2} \right)^2 \frac{1}{5 h^2}}$$

Calculations of $\bar{H}$ using this equation are given in the last column of Table 1; it is apparent that we may use this equation down to an h value of 3 cm without introducing an appreciable error in $\bar{H}$.

For a viscometer of the Fox, Fox, and FLORY type [9] the kinematical viscosity is given by

$$\nu = \alpha t - \frac{\beta}{t}$$

where

$$\alpha = \frac{\pi r^4 g \bar{H}}{8 Q (1 + nr)},$$

r is the radius of the capillary, nr is the end correction and Q is the volume of liquid discharged per second; β is a kinetic energy correction term. Thus α as determined experimentally should be directly proportional to the mean effective pressure head $\bar{H}$. In Fig. 3 a graph is given of α values plotted against $\bar{H}$ and h . With $\bar{H}$ as abscissa a good linear plot results passing through the origin, whilst using h the curve becomes non-linear at values of h less than 10 cm.

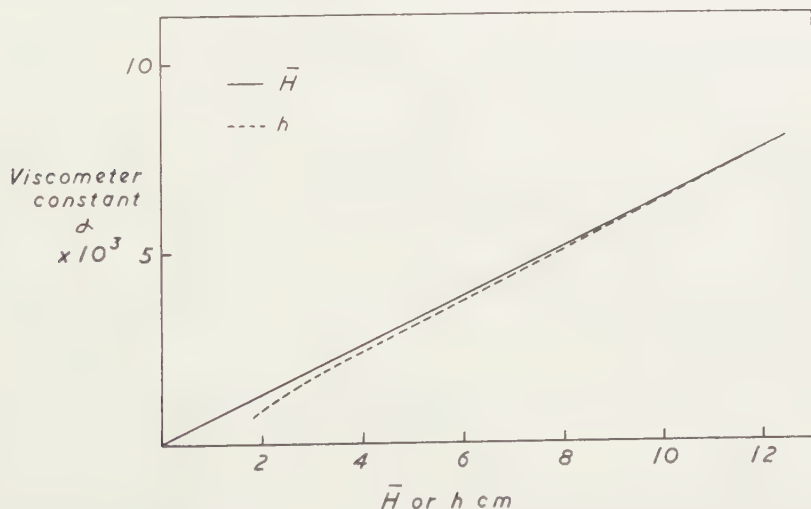


Fig. 3. Viscometer Constant α vs. Pressure head cm; ——— calculated values of mean effective pressure head $\bar{H}$, ---- arithmetic mean head h .

2b). Flow from a Sphere with Necks to a Cylinder

Since in viscometry we are usually dealing with a spherical bulb joined to a cylindrical tube at top and bottom, upon which the index marks are

engraved, this situation corresponds to the more practical case. Thus if the diameter of the spherical bulb is $2a$, the diameter of the associated cylinders with the index marks is $2c$ (see Fig. 2b) and the diameter of the inflow cylinder is $2b$, we have:

equations as for case 2a existing from $x = 0$ to $\sqrt{a^2 - c^2}$ and equations as for case 1 existing from $x = \sqrt{a^2 - c^2}$ to d , where d is the distance of the index mark above the centre of the sphere, whence

$$(10) \quad \int_0^d A \, dx = \pi \int_0^{\sqrt{a^2 - c^2}} (a^2 - x^2) \, dx + \pi \int_{\sqrt{a^2 - c^2}}^d c^2 \, dx = \pi [c^2 d + \frac{2}{3} (a^2 - c^2)^{3/2}]$$

From the calculations in the previous section it appears that the $1/h^2$ term in the correction factor is the most important and hence we have to evaluate the integral

$$\begin{aligned} \int_0^d A(x+y)^2 dx &= \pi \int_0^{\sqrt{a^2 - c^2}} (a^2 - x^2) \left(\frac{a^2 + b^2}{b^2} x - \frac{x^3}{3b^2} \right)^2 dx \\ &\quad + \pi \int_{\sqrt{a^2 - c^2}}^d c^2 \left(\frac{a^2 + b^2}{b^2} x \right)^2 dx. \end{aligned}$$

Thus putting

$$u = \frac{x}{a} \quad \text{and} \quad S = 3 \left(\frac{a^2 + b^2}{a^2} \right)$$

the solution is,

$$\begin{aligned} \frac{\pi a^9}{3^2 b^4} \left[\frac{S^2 u^3}{3} - \frac{2Su^5}{5} + \frac{u^7}{7} - \frac{u^5 S^2}{5} + \frac{2Su^7}{7} - \frac{u^9}{9} \right]_{u=0}^{u=\sqrt{1-c^2/a^2}} \\ + \frac{\pi c^2}{3} \left(\frac{a^2 + b^2}{b^2} \right)^2 [d^3 - (a^2 - c^2)^{3/2}]. \end{aligned}$$

If, as in the previous case, we retain for a first approximation only, the S^2 terms, this becomes,

$$\pi \left(\frac{a^2 + b^2}{b^2} \right) \left[\frac{c^2 d^3}{3} + \frac{2}{15} (a^2 - c^2)^{3/2} \right]$$

and we find,

$$(11) \quad \bar{H} = \frac{h}{1 + \frac{1}{5h^2} \left(\frac{a^2 + b^2}{b^2} \right) \left[\frac{5c^2 d^3 + 2(a^2 - c^2)^{3/2}}{3c^2 d + 2(a^2 - c^2)^{3/2}} \right]}$$

which when $c=0$ and $d=a$ becomes equal to equation (9A).

For the viscometer employed, with a and b as before, c was found to be 0.28 cm and $d=1.25$ cm and calculations of $\bar{H}$ using equation (11) yielded the values given in Table 2.

Comparison with Table 1 shows that under the conditions employed in this viscometer, i.e. with the index mark on the neck close to the bulb, then for all practical purposes, down to $h=5$ cm, we can neglect the

volume of the neck, and no serious error is introduced if we use the approximate equation (9A).

TABLE 2

Values of $\bar{H}$ after correction for the volume of liquid contained in the neck of the bulb

h cm	$\bar{H} = \frac{h}{1 + 1.5332/h^2}$
30	29.95
20	19.94
10	9.85
7.5	7.30
5	4.71
3	2.56
2	1.45

APPENDIX

An alternative approach to the solution of case 2a can be made by evaluating $\bar{H}$ in terms of the rise in level of the liquid in the cylinder. From Fig. 2a, we obtain that the unit of volume in the sphere dV is given by

$$dV = -\pi(a^2 - x^2) dx = \pi b^2 dy.$$

On integration between a and x , the rise in the cylinder is

$$(12) \quad y = \frac{(x-a)^2 (x+2a)}{3b^2}.$$

Note that y is counted upwards from the initial level and thus differs from the y shown in fig. 2a.

Then if f is the height between X and the initial level in the cylinder and H is the pressure head at time t ,

$$H = f + x - y$$

and

$$\frac{dV}{dt} = k(f + x - y)$$

giving on substitution for y from equation (12),

$$(13) \quad \frac{dV}{dt} = \frac{k}{3b^2} [(3b^2 f - 2a^3) + 3(a^2 + b^2)x - x^3].$$

Further, we can write

$$h = f - \frac{y_{\max}}{2}, \text{ and since } y_{\max} = \frac{4a^3}{3b^2}$$

when

$$x = -a, \quad f = h + \frac{2a^3}{3b^2}.$$

Thus by substitution for f in equation (13) and remembering

$$dV = -(a^2 - x^2)\pi dx,$$

$$\frac{dx}{dt} = \frac{-k}{3b^2\pi(a^2 - x^2)} (3b^2h + 3(a^2 + b^2)x - x^3)$$

whence the total time for flow from $x=a$ to $x=-a$ is

$$T = \frac{b^2\pi}{k} \int_{-a}^a \frac{3(a^2 - x^2)}{(3b^2h + 3(a^2 + b^2)x - x^3)} dx.$$

If the effective pressure head H is assumed to be the mean effective pressure head $\bar{H}$

$$\frac{dV}{dt} = kH$$

and

$$V = \int_0^T k\bar{H} dt = k\bar{H}T = \frac{4}{3}\pi a^3$$

giving

$$\bar{H} = \frac{4a^3 \cdot 3b^2}{\int_{-a}^a \frac{3(a^2 - x^2)}{3b^2h + 3(a^2 + b^2)x - x^3} dx}$$

or

$$(14) \quad \bar{H} = \frac{\text{Total rise in cylinder in cm}}{\int_{-a}^a \frac{3(a^2 - x^2)}{3b^2h + 3(a^2 + b^2)x - x^3} dx}.$$

Integration of equation (14) leads to the expression,

$$(15) \quad \bar{H} = \frac{\text{Total rise in cylinder in cm}}{\left[(1 - 2z) \ln(\sqrt{1-x} - x) + (1 - z) \ln(x^2 + \sqrt{1-x} + \beta) - \frac{6xz}{4\beta - x^2} \tan^{-1} \frac{2x + \sqrt{1-x}}{4\beta - x^2} \right]_a^{-a}},$$

where

$$z = \frac{b^2}{2(\sqrt{1-x} - a^2 - b)^2}.$$

This follows since the real root, $\sqrt{1-x}$, of the cubic expression in the denominator of the integral is given by

$$\sqrt{1-x} = \left(\frac{3b^2h}{2} + \sqrt{\left(\frac{3b^2h}{2} \right)^2 - (a^2 + b^2)^3} \right)^{\frac{1}{3}} + \left(\frac{3b^2h}{2} - \sqrt{\left(\frac{3b^2h}{2} \right)^2 - (a^2 + b^2)^3} \right)^{\frac{1}{3}}$$

and for the conditions used,

$$\frac{3b^2h}{2} = (a^2 + b^2)^{\frac{3}{2}}.$$

the expression has only one real root, and we may write the cubic as $(\alpha - x)(x^2 + \alpha x + \beta)$ with

$$\beta = \frac{3b^2h}{\alpha}.$$

Thus from equation (15) $\bar{H}$ can be calculated from the rise of liquid in the cylinder, which occurs as the liquid flows from one index mark on the bulb to the other. The rise can be measured accurately using a cathetometer and for the viscometer employed was found to be 1.98 cm. Using the values of a and b previously given the values of $\bar{H}$ were calculated for various values of h ; these are recorded in Table 3. It may be noted that equation (15) gives complete values for $\bar{H}$ and thus enables a direct check to be made of the errors involved in using curtailed series in equations (9) and (9A).

TABLE 3
Values of $\bar{H}$ calculated from equation (15)

h cm	$\bar{H}$ cm
30	29.96
10	9.92
5	4.81
3	2.68
2	1.35

Comparison of these values with those given in Table 1 shows that they are in excellent agreement with those given by equation (9), even down to $h = 2$ cm. At values of h greater than 3 cm the more approximate equation (9A) gives equally good results, and since this is much easier to use for computation it can be used in practice without loss of accuracy.

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ASTRONOMY

THE SPECTRUM OF THE ZODIACAL LIGHT

BY

J. M. BECKERS

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Introduction

During the last ten years much work has been done in order to acquire a better knowledge about the physical state of the interplanetary matter.

From intensity and polarization measurements of the zodiacal light (BEHR and SIEDENTOPF [1], BLACKWELL [2]) it was possible to determine the distribution of dust and free electrons in a layer around the ecliptic plane (see also ELSÄSSER [3]), assuming that the polarization is only due to the electron scattering of the sunlight.

FESSENKOV [4] tried to show that solid particles, scattering light, can also give an appreciable amount of polarization, reaching even 27 %, enough to explain the observed values. However, the last observations gave a much higher degree of polarization, up to 35 % (ELSÄSSER [5]). It will be very difficult to determine in this way the ratio: electrons: solid particles in the interplanetary matter, because of the unknown nature of the scattering dust. In any case it can be said that the values of Behr, Siedentopf and Blackwell give a maximum value for the electron abundance.

A study of spectra of the zodiacal light can give us further information, in the same way as it is the case for the outer corona, where it has been possible to determine from the central depth of fraunhofer lines and from equivalent widths the ratio $\frac{F_{\text{corona}}}{C_{\text{corona}}}$ and by this the electron abundance.

Observations

In order to obtain spectra of the zodiacal light many difficulties have to be overcome. It is necessary to work with a spectrograph of large aperture, and even then a long exposure time will be needed to give a spectrum of reasonable dispersion.

In addition to these difficulties, which we meet also in airglow spectrography, comes the short time of visibility of the zodiacal light. It is perhaps because of this that so few spectra are available. The spectra taken by E. A. FATH in 1907 [6] showed the G bands and the H and K lines; the last ones were not resolved by the instrument because of the large slitwidth. His conclusion was that the zodiacal light could be ascribed to scattered sunlight. More information can be obtained from

the spectra taken by HOFFMEISTER at the Windhuk station in South Africa [7].

He took several spectra of the airglow, the zodiacal light and the counter glow (Gegenschein) with a much higher resolving power than Fath did. In fig. 1 the microphotometer records of his spectra are republished, leaving aside most of the airglow spectra, which can be found in his publication. Clearly are seen the resolved H and K lines in the counter glow spectra (S 8 and S 12), while in the zodiacal light spectra (S 2, S 16, S 17 and S 34) they are present but no longer separated. In the spectrum of the airglow (S 18 and others not republished) the presence of these lines is very doubtful.

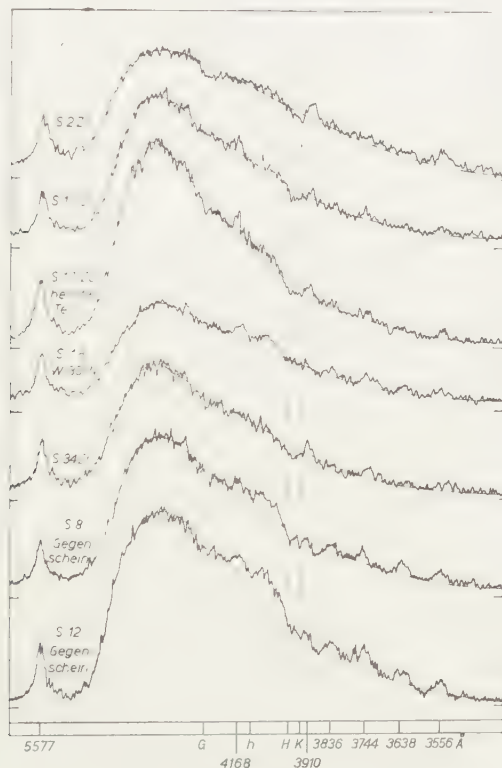


Fig. 1. Spectra of the zodiacal light and of the counter glow, taken by HOFFMEISTER [7]. The fully drawn line indicates the position of the undisturbed continuum, determined as well as possible from the windows between the absorption and emission lines.

However, according to CHAMBERLAIN, OLIVIER and MEINEL [8] the Ca II lines must be also present in the airglow spectra. They assume that the spectrum of the airglow is composed of two components: the emission line spectrum of the airglow proper and an absorption line spectrum of the stellar background. This last spectrum corresponds with the "mean" type of the stars, the Ca II lines being similar to those in FO type stars. In such stars the H and K lines are less strong than in the

sun; it is possible that they are washed out in Hoffmeister's spectra, while the solar H and K lines are still present.

The zodiacal light and counter glow will also contain starlight, but comparatively less than the airglow does. It is therefore reasonable to regard the line-spectra, which we shall discuss, as spectra of the scattered sunlight. We notice also the G band, already seen by Fath; the small dip near 4100 Å in the counter glow spectrum might be attributed to the H_δ line in the solar spectrum, but this is very doubtful because of the presence of neighbouring strong emission bands.

The very different appearance of the H and K lines in the different spectra can give important evidence about the nature of the interplanetary matter. The electrons around the sun have such a high temperature that the two lines in the scattered light melt together by doppler widening. The dust, however, will have much smaller thermal motions, which will not affect the line spectrum. A combination of these two components can perhaps explain the observations. Our calculations will be made in the assumption that the counter glow is caused mainly by solid particle reflexion, as it has been supposed already a long time ago (see e.g. H. WALTER [9]). The zodiacal light, however, will be assumed to contain an appreciable amount of light scattered by electrons. In the next chapters we shall first calculate how the H and K lines are deformed after scattering by electrons with a kinetic temperature T' . Then the line profile in the electron-part of the zodiacal light can be calculated by integration along the line of sight, assuming a density and temperature distribution for the gas in the planetary plane. This gives the possibility to make a quantitative analysis of Hoffmeister's spectra and to acquire more information about the ratio of electrons to solid particles and about the temperature distribution.

Transformation of the H and K line profiles by a Gaussian apparatus function

Starting from the Utrecht atlas of the solar spectrum, the spectral profile in the region of the H and K lines was slightly blurred, till the numerous weak lines disappeared. The deviations of this blurred profile from that given by Houtgast for the pure Ca II lines turned out to be very small.

It was now possible to represent the blurred profile by the sum of two Voigt profiles V_1 and V_2 with maxima at 3933.3 and 3968.3 Å, the constants of which are given in table I; they were determined by comparison with the table of G. ELSTE [10].

TABLE I

	K(3933.3)	H(3968.3)
β_1	5.69	4.88
β_2	2.32	0.00
h	12.64	7.96
a	16.875 Å	13.491 Å

As a preliminary to further considerations, we wish to investigate how these H and K profiles would be affected by a Gaussian apparatus function of the shape.

$$(1) \quad D(\Delta\lambda, T') = \frac{1}{7.25\sqrt{nT'}} e^{-\left(\frac{\Delta\lambda}{7.25}\right)^2 \frac{1}{T'}},$$

which is again a special case of a Voigt function.

The resulting combined profile can be obtained by blurring V_1 and V_2 by D and taking the sum of these blurred profiles. This can be done for various T' .

The figures 2 show the variation of the spectral intensity I relative to the continuum intensity for a number of selected wavelengths as a function of the T' . It appears that the two lines are separated for $T' < 10^5$; for higher T' the intensity at λ 3950 is lower than at λ 3933.3 and λ 3968.3. In the next section $I(\Delta\lambda, T')$ will denote this spectral profile as a function of T' .

The line profile in the electron part of the zodiacal light

We are now able to derive the spectral profile of the H and K lines in the light, scattered by the electrons inside a volume element on the line of sight. If the electrons themselves would emit these lines, the original spectral profile would be blurred by the apparatus function $D(\Delta\lambda, T')$ as explained before, T' being equal to the kinetic temperature T . Actually the electrons are scattering the sunlight and have also thermal motions relative to the sun. It is well-known that in such a case the whole blurring effect may be accounted for by a similar apparatus function as (1), if only T' is considered as a fictive temperature and is taken equal to $4T \sin^2 \frac{1}{2}\alpha = 2T(1 - \cos \alpha)$ (see *e.g.* UNSÖLD [12]; VAN HOUTEN [13]). The effect of such a blur is thus described by our function $I(\Delta\lambda, T')$, already represented in the graphs of fig. 2.

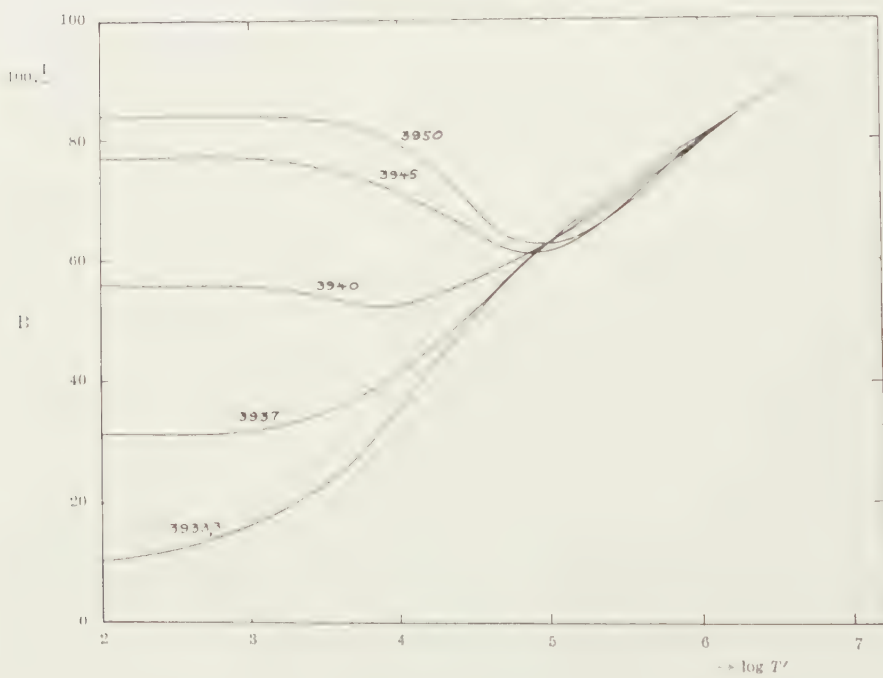
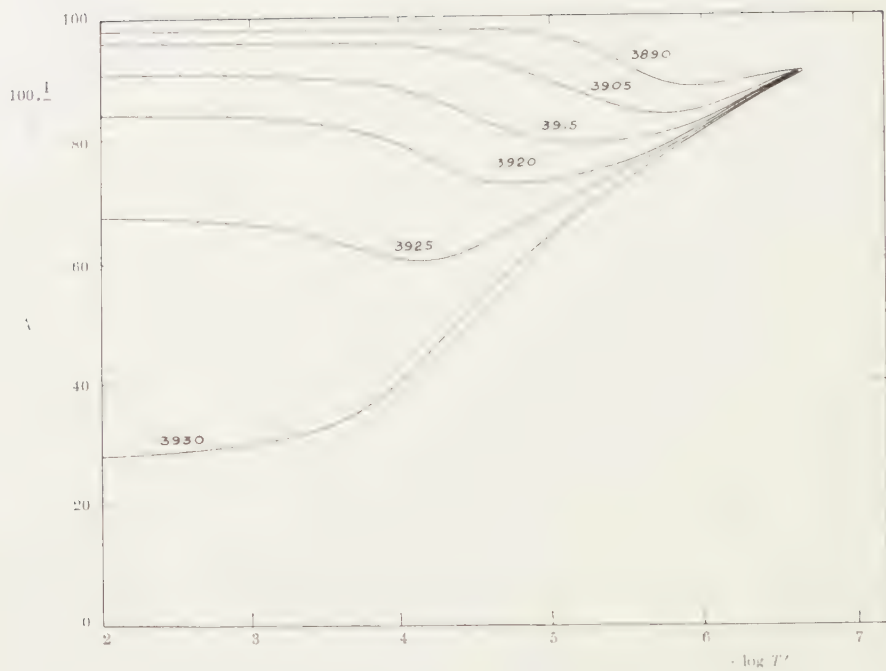
We shall now integrate along the line of sight the contributions of the different volume elements. For the continuous spectrum, according to ELSÄSSER [3], the surface brightness of a volume element of thickness dl , containing $n_e dl$ electrons, at a distance l from the Earth and r from the Sun, will be (see figure 3):

$$(2) \quad dJ_c = \frac{L_c R^2}{r^2} n_e(r) I(\alpha) dl,$$

where L_c is the surface brightness of the sun in continuous light near the H and K lines, R the distance Earth-Sun and $n_e(r)$ the electron density. $I(\alpha)$ represents the amount of scattering per steradian due to one electron, as a function of the scattering angle α ; it is equal to

$$(3) \quad \left(\frac{e^2}{mc^2}\right)^2 \frac{1 + \cos^2 \alpha}{2},$$

$$(4) \quad \text{of which } \left(\frac{e^2}{mc^2}\right)^2 \frac{\sin^2 \alpha}{2} \text{ is polarized.}$$



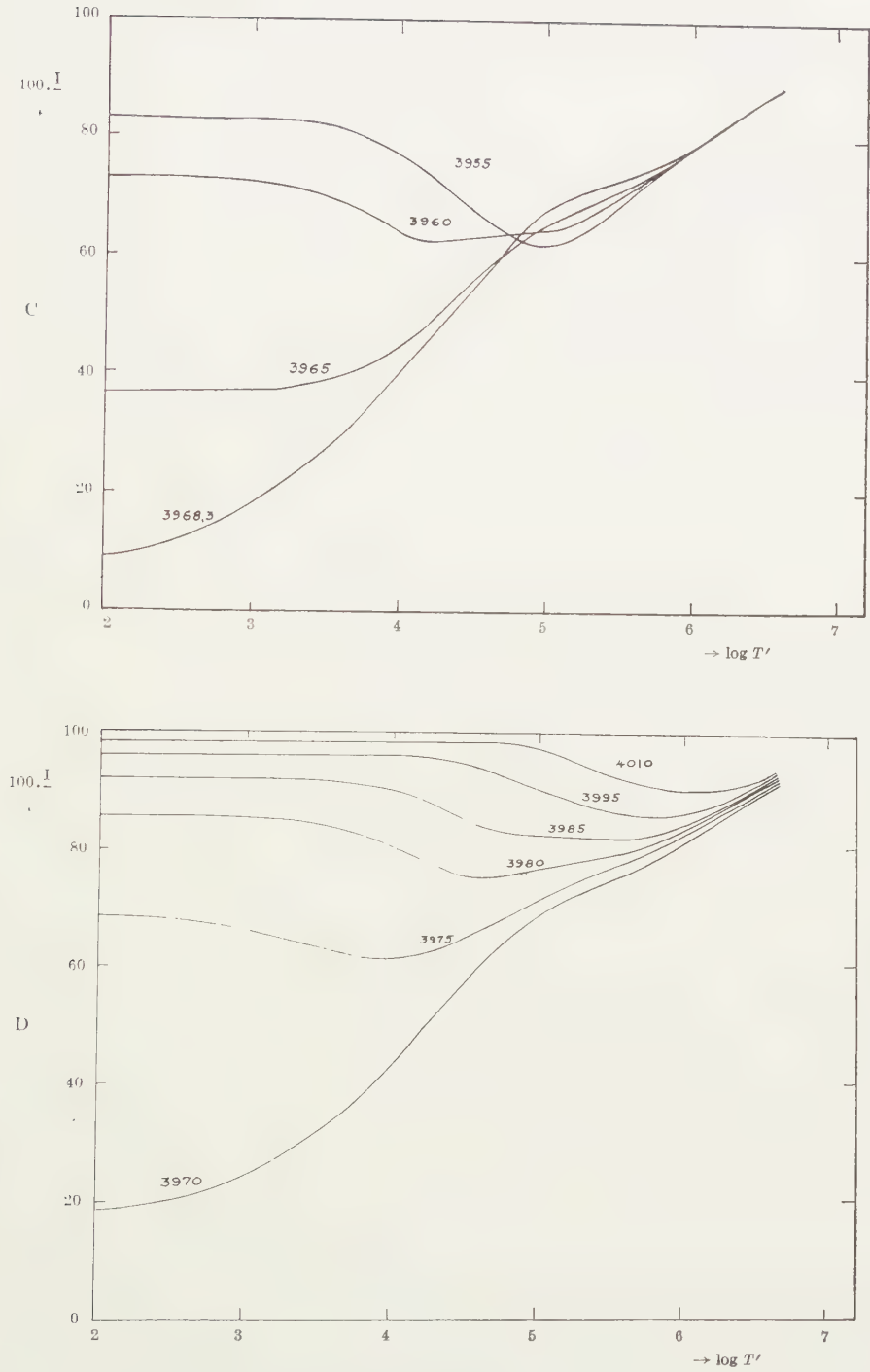


Fig. 2. The function $I(\Delta\lambda, T')$ which gives the variation of the depth in the H and K line profiles as a function of T' . The numbers in the diagram denote the different wavelengths in $\text{\AA}$.



Fig. 3

In order to find the spectral profile, the expression (2) has to be multiplied first by the function $I(\Delta\lambda, T')$; the product now has to be integrated along the line of sight. According to (2) and (3) the total brightness in unpolarized light is equal to:

$$(5) \quad J = \left(\frac{e^2}{mc^2}\right)^2 \frac{L}{2} \int_0^\infty \frac{R^2}{r^2} n_e(r) (1 + \cos^2\alpha) I(\Delta\lambda, T') dl$$

or, by using α as a variable instead of l (see fig. 3):

$$(6) \quad J = \left(\frac{e^2}{mc^2}\right)^2 \frac{LR}{2 \sin \xi} \int_{\frac{\pi}{2}}^{180^\circ} n_e(r) (1 + \cos^2\alpha) I(\Delta\lambda, T') d\alpha.$$

The intensity relative to the continuum intensity is

$$(7) \quad I_\xi(\Delta\lambda) = \frac{\int_{\frac{\pi}{2}}^{180^\circ} n_e(r) (1 + \cos^2\alpha) I(\Delta\lambda, T') d\alpha}{\int_{\frac{\pi}{2}}^{180^\circ} n_e(r) (1 + \cos^2\alpha) d\alpha}.$$

of which, by (2) and (4), the polarized fraction is found to be:

$$(8) \quad I_{\xi p}^{(\Delta\lambda)} = \frac{\int_{\frac{\pi}{2}}^{180^\circ} n_e(r) \sin^2\alpha I(\Delta\lambda, T') d\alpha}{\int_{\frac{\pi}{2}}^{180^\circ} n_e(r) \sin^2\alpha d\alpha}.$$

In order to compute these integrals we must make an assumption about the density and temperature distribution of the electron gas in the neighbourhood of the sun.

Density distribution

From observations of the polarized part of the zodiacal light a density distribution can be determined, assuming that the polarization is entirely due to the electrons.

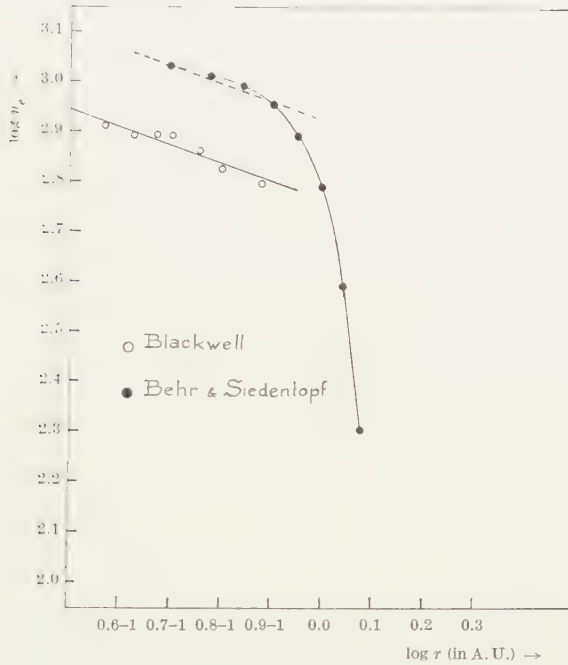


Fig. 4. Electron densities as a function of r according to [1] and [2]. The dashed line has the same slope as the line through Blackwell's observations.

Figure 4 represents the determinations of resp. BLACKWELL [2] and BEHR and SIEDENTOPF [1]. The values found by Blackwell can be very well described by:

$$n_e(r) = n_e(1)r^{-0.354}.$$

The values of Behr & Siedentopf satisfy this same equation fairly well for $r < R$, while for $r > R$ their density gradient is much higher. However, new observations [5] seem to give a considerably greater degree of polarization, especially for large distances from the sun, which would make the gradient smaller. Since, moreover, the final result of the calculation turns out to be very insensitive to the density distribution for $r > R$, it seems justified to use the density distribution (9) for the calculations.

Temperature distribution

From theoretical considerations about the thermal conductivity in a fully ionized gas, S. CHAPMAN [11] found that the temperature T in the planetary plane should vary according to the relation:

$$T(r) = T(1)r^{-3/7},$$

r being the distance to the centre of the sun. In order to determine the constant $T(1)$, we assume $T = 10^6$ °K at a distance $r = 1.06$ solar radii = 0.005 AU. Thus:

$$(10) \quad \log T = 5.341 - \frac{2}{7} \log \frac{r}{R}.$$

At the earth's distance, this would correspond to the surprisingly high temperature of 200,000 °K.

Integration

Using the functions $n_e(r)$ and $T(r)$ we are now able to carry out the integrations (7) and (8) for an angular distance ξ of the line of sight from the sun. The results of these integrations are given in table II.

TABLE II

λ	$\xi = 30^\circ$		$\xi = 50^\circ$	
	light	polarized part	light	polarized
3890 Å	90.9 %	90.6 %	90.2 %	90.4 %
3915	80.8	80.7	80.8	80.5
3933.3	73.8	74.2	75.0	74.2
3950	72.0	71.9	72.6	71.7
3968.3	76.0	74.9	75.5	74.9
3985	82.1	81.9	82.0	81.8
4010	90.9	90.6	90.3	90.5

It is striking that the profile of the line is practically independent of the ξ and of the polarization. In the next considerations the mean of the spectral distribution in total light is taken; we shall call it $W_1(\Delta\lambda)$.

Interpretation of the spectra

The observed spectral line is composed of the line due to the electrons $W_1(\Delta\lambda)$ and the line due to the dust $W_2(\Delta\lambda)$, which is similar to the original one.

Let x be the fraction of the light coming from the dust; then the composite profile will be:

$$W(\Delta\lambda) = (1-x)W_1(\Delta\lambda) + xW_2(\Delta\lambda).$$

By the apparatus function $A(\Delta\lambda)$ this will be changed into the observed profile:

$$S(\Delta\lambda) = (1-x)S_1(\Delta\lambda) + xS_2(\Delta\lambda),$$

S_i being equal to

$$\int_{-\infty}^{+\infty} W_i(\Delta\lambda) A(\Delta\lambda - \Delta\lambda') d(\Delta\lambda').$$

Taking for $A(\Delta\lambda)$ a rectangular profile, corresponding with an equivalent slit-width of 27.6 Å, we find for S_1 and S_2 the values of table III.

TABLE III

λ	S_1	S_2
3933.3 Å	74.5 %	52.9 %
3950	73.1	71.0
3968.3	76.2	58.9

The spectral profile $S(\Delta\lambda)$ can now easily be computed for different values of x (figure 5a).

In Hoffmeister's spectra the peak near 3950 Å has disappeared; therefore x must be ≤ 0.4 . This is, however, in contradiction with the earlier determinations, [1], [2] and [3] were $x=0.6$, or even greater if Fessenkov's theory is true.

The counterglow profile will be the same as S_2 , because $x=1$ according to WALTER [9]; the electron zodiacal light would not show such a sharp maximum under a scattering angle of 180°, it is therefore reasonable to ascribe the counterglow to dust. Thus in Hoffmeister's counterglow

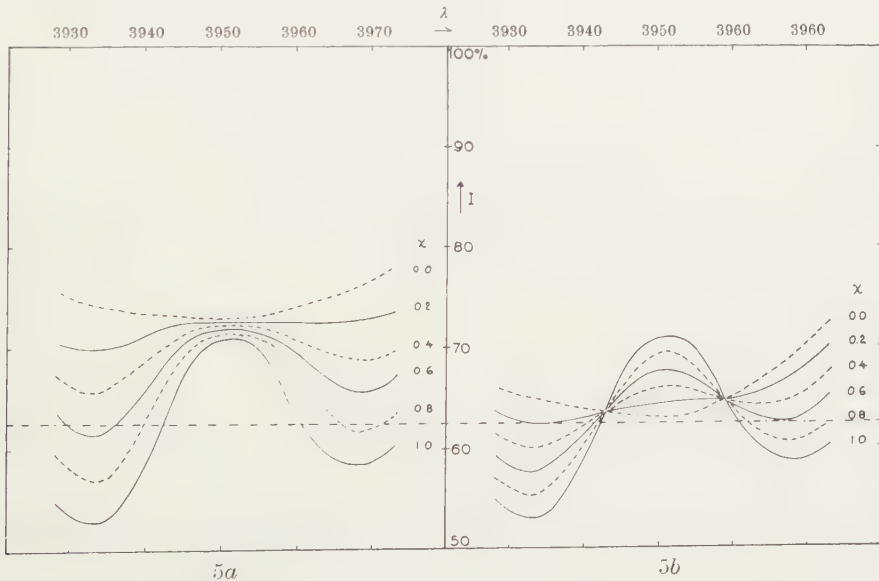


Fig. 5a. Computed line profiles $S(\Delta\lambda)$ for different x and for a temperature distribution according to Chapman. The dash-dotted line denotes the measured depth of the zodiacal H and K lines.

Fig. 5b. Computed line profile $S'(\Delta\lambda)$ for a temperature about 5 times lower than that of Chapman.

spectra the corresponding intensities are known; using this it is possible to calibrate his zodiacal light spectra. This gives a central intensity for the H and K lines of: $62.5 \% \pm 4 \%$, also in contradiction with the theoretical values.

A lower temperature of the electron cloud could give a better agreement. The profile $W_1(\Delta\lambda)$ is found to be nearly the same as if the whole of the electrons were at an intermediate effective temperature corresponding to $\log T' = 5.69$. Thus $W_1(\Delta\lambda) = I(\Delta\lambda, [5.69])$.

Let us consider a lower temperature and take for example the profile belonging to $\log T' = 5.00$. Without repeating a new integration with a temperature model we write: $W_1(\Delta\lambda) = I(\Delta\lambda, [5.00])$.

Figure 5b shows the new $S'(\Delta\lambda) = (1-x)S'_1(\Delta\lambda) + xS_2(\Delta\lambda)$ as a function of x . The central depth agrees better, but a determination of x gives now $x \leq 0.5$, still too small with regard to the other values.

A still lower value of T would give again a smaller x because in the $W_1(\Delta\lambda)$ spectrum the peak at $3950 \text{ \AA}$ between the two lines is then again present. — Of course one has to regard these results with much caution. The spectra of Hoffmeister are not calibrated and their resolving power is small. The disturbing emission line of the airglow on the violet side of the K line may deform the H and K line profile. Better observations can give us perhaps more information about the nature of the zodiacal light. The depth of not too strong Fraunhofer lines as *e.g.* H_δ or others, which must have totally disappeared in the spectrum of the electron light, is then a measure for the ratio electron-dust. The strong lines, in the first place the H and K lines, give more information about the temperature.

Provisionally we can conclude that the polarization is mainly due to the electrons and not to the dust as FESSENKOV assumed; and that the temperature of the planetary electron gas in the neighbourhood of the earth is about one fifth of that, found by S. CHAPMAN.

The equation $T(r) = T(1)r^{-1}$ would represent better the temperature variation with the distance to the sun, giving at the earth's distance $40,000^\circ \text{K}$.

Addendum

Up till now we assumed that the interplanetary gas is a stationary cloud of ions and electrons. However, JOHNSON [14] suggested, that the corpuscles of the zodiacal light, moving away from the sun, could modify the spectrum of the reflected light because of their outstreaming motion. They have radial velocities with respect to the earth, which can be both positive ($\alpha > 90^\circ$ in fig. 3) and negative ($\alpha < 90^\circ$). Combined with their motion with respect to the sun, this causes a displacement of the lines to the red but also a widening of the lines, and gives perhaps another possibility of explaining the blurred H and K profiles. By observing their wavelengths it must be possible in principle to decide, whether the interplanetary gas is stationary or not.

JOHNSON and HOFFMEISTER did not observe a shift of the H and K lines in the counter glow; thus the radial velocity of the counter glow particles (probably dust) is < 150 km/sec as it was to be expected. The available zodiacal light spectra are the sum of a dust and an electron component, which cannot well be separated and of which the last one is deformed by the widening function.

This widening function has been calculated for the already assumed density distribution: $n_e(r) = n_e(1)r^{-0.354}$ and for two velocity distributions $v(r)$.

According to equation (2) the total brightness in unpolarized light will be (regarding the continuum only):

$$J_c = \left(\frac{e^2}{mc^2} \right) \frac{L_c R^2 n_e(1)}{2} \int_0^{\infty} \frac{1 + \cos^2 \alpha}{r^{2.354}} dl$$

The function $\frac{1 + \cos^2 \alpha}{r^{2.354}} = F_1(l)$ gives the relative importance of the contribution to J_c from the electrons at a distance l from the earth.

Assume $v(r)$ is equal to $v(r) = v(1)r^{-a}$; then the radial velocity $v_r(l)$ in a point at a distance l is equal to:

$$v(r) \cdot (1 - \cos \alpha) = v(1) \cdot r^{-a} \cdot (1 - \cos \alpha)$$

With a certain distance $\Delta v_r(l)$ will correspond an interval Δl and with this a contribution to the total light received on earth, proportional to $F_1(l)\Delta l$.

So $\frac{v(1)F_1(l)\Delta l}{\Delta v_r(l)} \equiv N(v_r)$ gives the relative importance of the radial velocity v_r in the scattered sunlight. Would the sun emit only an emission line of infinitely small width, then the shape of the line profile in the scattered light would correspond with: $N(v_r) = N(c \cdot \Delta \lambda / \lambda)$. This function of $\Delta \lambda$ can thus be handled as an apparatus function. After smoothing the sun's spectrum with it we shall obtain the spectrum of the scattered light. This $N(v_r)$ has been calculated for two different velocity distributions, viz. $a = 1.646$ and $a = 0$, corresponding resp. with $n(r) \cdot r^2 \cdot v(r) = \text{constant}$ (as it is the case when the flow of matter around the sun is radial), and with a constant outward stream velocity. The resulting functions are shown in fig. 6a and 6b. In fig. 6a the function $N(v_r)$ is very uncertain for $v_r < 0.4 v(1)$, because of (1) difficulties arising in the numerical method used for deriving the function; and (2) the uncertain extrapolations in the electron density for $r > 1$ AU. The calculations have been made for a distance from the sun $\xi = 50^\circ$.

For the case that $a = 1.646$ one can say that in first approximation the line is splitted into two components, one with a wavelength shift of about zero and another with a shift equal to about $(1.8 v(1) \lambda / c)$ Å. Probably the H and K lines will no longer be separated, if the red shift of the second component of the K line is about 18 Å, which corresponds to $v(1) = 750$ km/sec. This very rough estimate gives a not improbable value for the

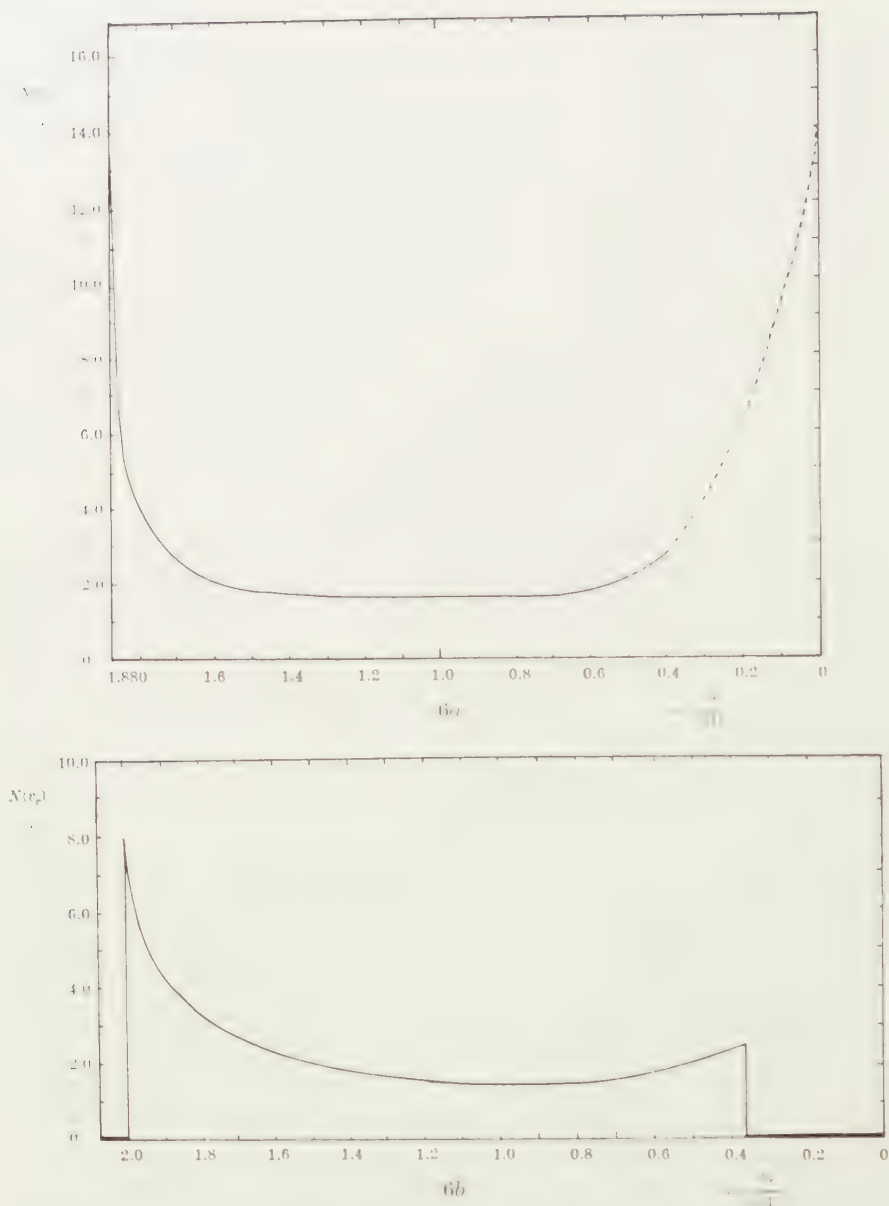


Fig. 6. The function $N(v_r) = N\left(\frac{c \cdot d\lambda}{\lambda}\right)$ resp. for $a = 1.646$ and for $a = 0$.

outward stream velocity of the gases near the earth. The case $a = 0$ would give a slightly higher value.

We conclude that Johnson's suggestion gives another possibility to explain the observed spectra. Careful observations of line profiles, including the weaker Fraunhofer lines, can give an answer to the following questions:

1°) Is the zodiacal gas stationary or streaming outward? In the second case there is a splitting of the weaker lines or a displacement, if the two line components cannot be observed separately.

2°) If the gas is stationary, what is its temperature? This can be obtained from the H and K line profiles.

3°) If the gas is stationary, the ratio dust-electrons can be found from the depth of the weakened Fraunhofer lines; they have completely faded out in the electron component. If the gas is only streaming outward one can get information from the spectrum of the polarized part of the zodiacal light. This part is mainly due to gases at $\alpha = 90^\circ$ (nearest to the sun). This gives only a shift of the line, not a splitting. The non-displaced line is due to the dust and the displaced one to the electrons.

The ratio dust-electrons, determined in this way, gives a possibility to determine the degree of polarization of the dust; so we can get an idea of its properties.

The following additional references [15] to [18] complement our list of zodiacal light spectra.

Summary

Hoffmeister's spectra of the zodiacal light and of the airglow are discussed. The appearance of the H and K lines in these spectra may be accounted for, if the zodiacal light is interpreted as sunlight, scattered by stationary interplanetary dust and electrons. The temperature distribution in interplanetary space according to CHAPMAN cannot explain the observations; a temperature distribution of the form $T(r) = T(1)r^{-1}$ is more probable. We find that 50 % or more of the zodiacal light is due to electron scattering. From polarization observations previous authors derived, that 40 % is due to electrons, and this fraction would be even less, if Fessenkov's suggestion about the polarization of dust-scattered light were true; on the basis of this assumption it would be difficult to reach agreement between the results of spectral observations and those of polarization measurements.

Resumo

En spektroj de la zodiaka lumo, fotografitaj de HOFFMEISTER, la H-kaj K-linioj ne estas apartaj, dum ili ja estas tiaj en la spektroj de la "Gegenschein". Ĉi tion oni povas klarigi, se oni supozas ke grava parto de la zodiaka lumo estiĝas pro difuzo far elektronoj; ties varmoviĝado konfuzas la spektroliniojn. Per la kalkulo de la ekspektenda liniprofilo estas montrate ke la absoluta temperaturo de la interplaneda gaso devas esti proksimume $1/5$ de tiu kiun supozis CHAPMAN. Minimume 50 % de la lumo devenas de farelektrona difuzo. La lumo de la "Gegenschein", kontraŭe, devenas de polverkaŭsata difuzo. — La grava plilarĝiĝo de la H- kaj K-linioj povus esti klarigata ankaŭ per la supozo ke la difuzantaj korpuskloj moviĝas for de la suno kun rapido de minimume 750 km/sek.

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CONTENTS

Astronomy

BECKERS, J. M.: The spectrum of the zodiacal light. (Communicated by Prof. M. G. J. MINNAERT), p. 248.

Biochemistry

HOOGHWINKEL, G. J. M., J. TH. HOOGEVEEN, M. J. LEXMOND and H. G. BUNGENBERG DE JONG: Spot tests for phospholipids and their use in paper chromatography, p. 222.

Chemistry, Colloid

BUNGENBERG DE JONG, H. G. and J. TH. HOOGEVEEN: Hydrosols of total egg-phosphatides. Ia, p. 201.

BUNGENBERG DE JONG, H. G. and J. TH. HOOGEVEEN: Hydrosols of total egg-phosphatides. Ib, p. 214.

Chemistry, Physical

OTTEWILL, R. H. and J. TH. G. OVERBEEK: On the calculation of the mean effective pressure head for capillary viscometers, p. 236.